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# Towards a Better Understanding of Nonstructural Carbohydrate Storage and Carbon Limitation in Trees

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**Abstract**
Nonstructural carbohydrates (NSC) are an important component of the carbon storage pool in trees and commonly used to assess whether tree growth under various conditions is carbon limited. However, we know very little about what causes variation in NSC levels and allocation among trees. While NSCs may accumulate passively when growth is carbon saturated, their storage may be active, competing with growth for carbon. If storage is mostly an active process, trees may maintain high NSC levels at the expense of potential growth, making NSC levels poor indicators of carbon saturated growth. To determine how growth and NSC allocation are related, we compared variation in the seasonal changes in NSC levels (NSC increment) with variation in radial growth in mature, canopy black oaks in the New Jersey Pine Barrens. Using experimental defoliation of saplings in a common garden and mature trees in the field, we also explored whether allocation shifts that favor storage reduce growth.

Growth and NSC increment were positively correlated, indicating that storage was active, not the result of carbon-saturated growth. Defoliation reduced sapling growth, but increased the proportion of biomass allocated to NSCs. In adults, defoliation reduced growth for three years. However, while aboveground NSC levels may not have completely recovered by the end of the third year, belowground levels recovered after two years due to greater NSC allocation. Defoliation also changed the relationship between NSC increment and growth in the two years following defoliation, causing a shift in allocation that favored storage. The positive relationship between growth and NSC increment indicated that the allocation shift was not due to sink limitation. We conclude that storage is a high priority in these trees. In addition, carbon limiting events like defoliation can greatly alter carbon allocation by increasing the priority of storage, potentially in response to greater threats of carbon starvation. Our results indicate that carbon limitation to growth may occur not only because of decreased carbon availability but because of allocation shifts that reduce the priority of growth relative to other processes.

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# TOWARDS A BETTER UNDERSTANDING OF NONSTRUCTURAL CARBOHYDRATE STORAGE AND CARBON LIMITATION IN TREES

Erin Wiley

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# ABSTRACT

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Nonstructural carbohydrates (NSC) are an important component of the carbon storage pool in trees and commonly used to assess whether tree growth under various conditions is carbon limited. However, we know very little about what causes variation in NSC levels and allocation among trees. While NSCs may accumulate passively when growth is carbon saturated, their storage may be active, competing with growth for carbon. If storage is mostly an active process, trees may maintain high NSC levels at the expense of potential growth, making NSC levels poor indicators of carbon saturated growth. To determine how growth and NSC allocation are related, we compared variation in the seasonal changes in NSC levels (NSC increment) with variation in radial growth in mature, canopy black oaks in the New Jersey Pine Barrens. Using experimental defoliation of saplings in a common garden and mature trees in the field, we also explored whether allocation shifts that favor storage reduce growth.

Growth and NSC increment were positively correlated, indicating that storage was active, not the result of carbon-saturated growth. Defoliation reduced sapling growth, but increased the proportion of biomass allocated to NSCs. In adults, defoliation reduced growth for three years. However, while aboveground NSC levels may not have

completely recovered by the end of the third year, belowground levels recovered after two years due to greater NSC allocation. Defoliation also changed the relationship between NSC increment and growth in the two years following defoliation, causing a shift in allocation that favored storage. The positive relationship between growth and NSC increment indicated that the allocation shift was not due to sink limitation. We conclude that storage is a high priority in these trees. In addition, carbon limiting events like defoliation can greatly alter carbon allocation by increasing the priority of storage, potentially in response to greater threats of carbon starvation. Our results indicate that carbon limitation to growth may occur not only because of decreased carbon availability but because of allocation shifts that reduce the priority of growth relative to other processes.

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# Introduction

Every year, forested ecosystems take up nearly 60 Pg of carbon, approximately half of terrestrial gross primary productivity (Beer et al. 2010). Knowledge of carbon allocation decisions in trees, as the dominant component of these systems, is therefore extremely important for understanding and predicting the fate of one the largest fluxes in the global carbon cycle. Within a tree, carbon may be allocated to several different processes including respiration, storage, reproduction, defense, root exudation, production of volatiles, and growth. Allocation to growth, especially of woody tissue, is of particular interest as it can sequester carbon from the atmosphere for hundreds of years while a tree lives and eventually can contribute to the soil organic pool, where carbon can be stored for 1000s of years. As climate changes, understanding how tree growth and allocation in general will change is critical for predicting whether forests will be sinks (i.e. entities that take up more than they release) or sources of atmospheric carbon. As sinks, forests can mitigate further climate change; as sources, they can exacerbate it.

Forests have already reduced rising $CO_2$ levels from anthropogenic carbon emissions, in part due to enhanced tree growth (Myneni et al. 2001, Luyssaert et al. 2008, Pan et al. 2011), but it is not clear if tree growth will continue to be stimulated. A major source of uncertainty is our poor understanding of when and where tree growth is carbon limited. Carbon limitation occurs when growth is limited by the availability of carbon for growth. If growth is instead carbon saturated, then trees are said to be sink limited. Sink limitation occurs when growth is limited by a tree's ability to use the available carbon for

growth due to such factors as insufficient nutrient supply, direct environmental constraints on the rate of biosynthesis (e.g. temperature), or developmental constraints (i.e. architectural constraints or phenology). Under conditions where trees are carbon limited, elevated $CO_2$ levels may enhance tree growth and forest carbon sequestration. If trees are sink limited, elevated $CO_2$ levels will have no effect.

The extent to which tree growth is carbon limited has been intensely debated over the past two decades. Disagreement extends from tropical rainforest (e.g. Clark 2004, Lloyd and Farquhar 2008) to alpine treeline (Stevens and Fox 1991, Korner 1999, Bansal and Germino 2008), from the effects of water stress induced by drought or tree height (Sala and Hoch 2009, Drake et al. 2010). Results of Free Air CO2 enrichment (FACE) experiments have also been ambiguous. Tree growth is often stimulated (Delucia et al. 1999, Hattenschwiler et al. 2002, Liberloo et al. 2006, Norby et al. 2010, Dawes et al. 2013), but this stimulation can diminish or disappear after several years, as nitrogen becomes limiting (Oren et al. 2001, Norby et al. 2010). Also, the response to elevated CO2 may be species specific (Handa et al. 2005, 2006), with some species responding and others exhibiting no response at all. In addition, these experiments tend to use young stands. The one experiment that has looked at mature tree growth detected no aboveground stimulation (Korner et al. 2005). At the same time, photosynthesis tends to be and to remain stimulated by higher CO2 (Ainsworth and Long 2005), but the fate of this additional carbon is often unclear, again highlighting our poor understanding of allocation in trees.

In part because of the high costs, technical difficulties, and limited spatial scale of FACE experiments, nonstructural carbon levels, particularly of nonstructural carbohydrates (NSC), in plants is often used as an indicator of tree growth limitation. Low or reduced levels are thought to be indicative of carbon limitation, while higher levels are thought to be indicative of sink limited growth (Korner 2003). From seasonal patterns of NSC levels and comparisons between sites of known tree growth differences, many have concluded that there is little leeway for growth enhancement from rising $CO_2$ levels (Korner 2003, Millard et al. 2007).

*Nonstructural carbohydrates*

NSCs are carbohydrates that can be remobilized for use in a variety of plant processes. As major components of the carbon storage pool, NSCs can provide a temporary source of carbon when current photosynthesis cannot meet the immediate carbon demands of the plant. Once mobilized, NSCs may be used to support metabolism, structural growth, defense, and reproduction. In addition, NSCs can be used to maintain a plant's hydraulic integrity (Woodruff and Meinzer 2011, Sala et al. 2012) via cryoprotection (Tinus et al. 2000), turgor maintenance and osmoprotection (Ingram & Bartels, 1996; Sanchez et al., 1998), and embolism repair (Bucci et al., 2003, Améglio et al., 2004).

NSC storage is relevant to many areas of tree ecology. First, NSC dynamics may help to explain patterns of tree diversity and the distribution limits of tree species and of the tree form. NSC levels or pool size often correlate with survival or disturbance

recovery (Webb, 1981; Canham et al., 1999; Gleason & Ares, 2004; Bréda et al., 2006; Myers & Kitajima, 2007; Galiano et al., 2011), but allocating carbon to storage potentially reduces carbon immediately available for growth. Therefore a tradeoff between growth and storage may affect the evolution of allocation decisions and life history strategies in trees (Poorter & Kitajima, 2007; Hultine et al. 2013), and this tradeoff may in part explain the tradeoffs between growth rates and survival (Pacala et al. 1994, Kobe et al. 1995, Seiwa 2007) or between competitive ability and stress tolerance (Grime 1977).

NSCs also play an important role in the carbon cycle at the ecosystem level. NSC storage can act as a buffer for net primary production (NPP; gross primary production or photosynthesis less plant respiration), explaining the imbalance between net carbon uptake and NPP either seasonally or between years (Gough et al., 2009; Richardson et al., 2013). Modeling carbon fluxes at the ecosystem level therefore requires an understanding of when and how much trees allocate to NSC storage, but storage allocation remains one of the most poorly modeled carbon pools in trees (LeRoux 2001). In addition, NSC dynamics are now commonly used to determine if tree growth is carbon limited, and therefore are relevant to predicting a forest's carbon sequestration potential.

*Storage versus growth*

The ecological importance of the NSC storage pool is largely due to its relationship with growth, but this relationship is poorly understood. In general, NSC storage may be classified in two ways, depending on how it affects growth. NSCs may

accumulate passively when growth is carbon saturated, or they may build up actively while growth is still carbon limited (Chapin et al. 1990). It is active storage which will play a role in any tradeoff between growth and storage processes, and as will be argued in Chapter 1, is problematic for the current use of NSC dynamics as an indicator of growth limitation.

The purpose of this dissertation research was to improve our understanding of the relationship between growth and NSC storage and of how NSC dynamics relate to carbon limitation. Using mature trees and saplings of *Quercus velutina* (black oak) as a model, we explored how carbon limitation affects the relationship between growth and storage via experimental defoliation. While many studies have documented seasonal and environmental changes in NSC and growth, exploring between-site or between-treatment variation, these studies almost always ignore the substantial within-site or within-treatment variation among individual trees. We have taken advantage of this variation to determine how growth is related to NSC storage and how defoliation affects this relationship. Our overarching hypotheses were that NSC storage is largely an active process that can limit growth and that carbon-limiting stresses may cause NSC allocation to increase relative to growth. If true, inferring sink limited growth from high or increased NSC levels is not correct, and carbon limitation may be much more common than currently believed.

In chapter one, we begin by arguing that inferring sink limitation from increased NSC levels is problematic because it relies on the assumption that storage is both passively formed and maintained. We also argue that carbon limitation may occur not

only as the result of reduced photosynthesis but in response to changes in allocation priorities. In chapter two, we use two levels of defoliation in a common garden setting to test if NSC levels can be maintained or increased when growth becomes (more) carbon limited. In chapters three and four, we explore the relationship between NSC dynamics and growth among mature trees in the New Jersey Pinelands and build a framework to better understand how growth limitation is related to this relationship. Finally, we use full canopy defoliation to test if shifts in allocation, in addition to reduced carbon uptake, can increase carbon limitation in trees.

# CHAPTER 1: A RE-EVALUATION OF CARBON STORAGE IN TREES LENDS GREATER SUPPORT FOR CARBON LIMITATION TO GROWTH

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We still have a poor understanding of what limits tree growth under many conditions. In general, tree growth can be limited by the *availability* of carbon within the plant (carbon limitation) or by the tree's *ability to use* the available carbon it has because of nutrient shortage, environmental conditions, or developmental constraints (sink limitation).

A growing body of literature suggests that current tree growth is sink limited under most conditions, with much of the evidence inferred from trees' high levels of nonstructural carbon (NSC). These inferences rely on the assumption that carbon storage is passive and that NSC does not accumulate when growth is carbon-limited (Bansal & Germino, 2008). However, storage may be an active process, occurring at the expense of growth (Chapin et al., 1990; Lacointe et al., 2004; Genet et al., 2010).

We argue that carbon starvation— even if it does not occur every year or even every decade in the life of a tree— is crucial to understanding carbon storage and carbon limitation in trees. (We define carbon starvation, modified after McDowell (2011), as the outcome of insufficient carbon supply from current photosynthesis and available storage to sustain the metabolism, regeneration, or defense necessary for immediate survival.) Episodes of carbon starvation (past and present) may have led to the evolution of conservative allocation strategies favoring greater reserve formation at the expense of

potential growth. Therefore, an increase in carbon storage and decrease in growth could be a plastic or evolutionary response to carbon-limiting conditions—not a result of sink limitation. To illustrate this point, we reexamine two phenomena relevant to the active debates regarding carbon limitation, tree growth at alpine treeline and under water stress.

**Storage and growth limitation**

Carbon storage, defined here as the formation of nonstructural carbon compounds that "can be mobilized in the future to support biosynthesis" can be divided into two very different processes: passive and active (Chapin et al., 1990). *Passive storage* forms an excess pool when carbon supply is greater than carbon demand of other processes, such as growth (See Figure 1.1). *Active storage, or reserve formation,* on the other hand, occurs when carbon is directed into storage *at the expense of growth or other processes*. Reserve formation allows a tree to prepare for (1) predictable asynchrony of supply and demand for carbon (e.g. early spring growth for deciduous trees) and (2) unpredictable emergencies such as disturbances that affect carbon gain or need (Chapin et al., 1990).

The potential for active reserves is often ignored when inferring the nature of limitation. When comparing trees in different sites or treatments, sink limitation to growth is inferred from one of two main patterns: (1) higher NSC content together with lower growth rates (Hoch & Körner, 2003; Würth et al., 2005; Palacio et al., 2008; Sala & Hoch, 2009), or (2) relatively stable NSC levels throughout the year that do not decline to zero (Hoch et al., 2002; Hoch et al., 2003; Shi et al., 2008). The rationale for inferring

sink limitation from the first pattern is that NSC pool size is the difference between carbon gain (source) and carbon demand (sink) within the plant (Körner, 2003). This assumption is true if all storage ispassive, but not if it is active, reserve carbon (Lacointe et al., 2004, Bansal & Germino, 2008, Genet et al., 2010). Reduced growth and increased storage is also consistent with carbon limitation if (a) populations vary genetically in the amount of active reserve formation or (b) reserve formation responds plastically to environmental or internal cues. The rationale for inference (2) is that if growth is annually carbon limited during periods where demand exceeds supply, NSC should approach zero during these periods, because growth could only be carbon limited if all storage is depleted (Hoch et al., 2003; Körner, 2003). But this complete, yearly drawdown will only occur if all reserves are stored because of annual asynchrony of supply and demand (i.e. earmarked for a particular time of year or frequent event). Reserves, though, can be formed to avoid running out of carbon during emergencies, which may be unpredictable and infrequent, and trees may actively maintain a baseline level of NSC, growing only after storage requirements are met.

**Increased storage increases survival but reduces growth**

Growth in trees with higher NSC levels may be more carbon limited than in those with lower NSC levels. Increased storage is often associated with survival (Canham et al., 1999; Gleason & Ares, 2004, Myers & Kitajima, 2007; although see Piper et al., 2009), recovery from disturbance (Breda et al., 2006), or resistance to biotic attack (Dunn et al., 1987). If storage can be active and increase the chance of survival,

then *increasing* storage could be an adaptive response to an increased threat of carbon starvation. Populations that experience severe episodes of carbon starvation may experience stronger selection for increased reserves, leading to more conservative allocation strategies. However, these conservative populations, while less prone to carbon starvation, are more likely to experience carbon limitation to growth because less carbon is available after reserves are formed. Therefore higher NSC levels may indicate, and cause, greater carbon limitation.

It has been argued that large reserves cannot be beneficial given (1) the large opportunity costs of not growing, and (2) that maintaining high photosynthesis rates necessary to develop such reserves is costly, in terms of nitrogen and phosphorus, and risky, in terms of cavitation (Sala & Hoch, 2009), but maintaining substantial reserves may have large payoffs. If a rare drawdown of storage occurs (due to a severe disturbance), a tree that has not saved enough to survive will be at a huge fitness disadvantage compared to surviving trees that continue reproducing for potentially hundreds of years. Selection for allocation patterns that minimize the risk of carbon starvation could easily lead to the emerging pattern of large carbon reserves that are never depleted within a season (as reported in Hoch et al., 2003; Körner, 2003).

Additionally, if carbon starvation is infrequent but relatively predictable by a reliable cue, plasticity of reserve allocation may be selected for. For example, when carbon uptake is low, a tree will take longer to accumulate enough carbon to recover from a disturbance or defend against biotic attack unless it has sufficient reserves; therefore storing more when carbon uptake declines could help a plant survive or recover faster.

Non-carbon status cues (such as temperature or water potential) could also be reliable indicators of increasing threat of starvation or damage, against which an increase in storage could defend. In *Arabidopsis thaliana,* there is accumulating evidence that plants actively shift carbon allocation between storage and growth, favoring storage when carbon uptake declines (Smith & Stitt, 2007; Gibon et al., 2009). Growth slows under salt stress and at cold temperatures in part due to higher levels of DELLA proteins, which down-regulate growth (Achard et al., 2006; 2008). Mutants without these proteins grow faster and have lower survival than wildtype plants under salt stress, indicating that growth is actively, and potentially adaptively, suppressed. . Suppression may be the way plants switch to a more conservative strategy of carbon use in response to cues from the environment to mitigate the effects of harsh conditions (Smith & Stitt 2007).

## Re-examining purported sink limiting cases

### *Treeline*

NSC levels are often used to support the growth-limitation hypothesis, which predicts that the absence of trees above treeline is due to direct inhibition of growth by cold temperatures (Körner, 1998). The main rationale for this hypothesis is that cell division slows faster than photosynthesis as temperatures decline (Körner, 1999). If trees cannot build new tissues fast enough to keep pace with photosynthesis, carbon substrates will build up in the plant; alternatively, if carbon supply were limiting, NSC concentrations would be lower at treeline (Hoch et al., 2002). Treeline trees often grow

slower and have higher NSC concentrations than trees at lower altitudes, presumably supporting the growth limitation hypothesis (Hoch et al., 2002; Hoch & Körner, 2003; Shi et al., 2008).

However, because increased storage may be adaptive and actively controlled (Sveinbjörnsson, 2000; Bansal & Germino, 2008), observed patterns in growth and storage are also consistent with avoidance of carbon starvation. Active storage can account for decreased growth and increased NSC at treeline in two ways. (1) Increased storage may represent a local adaptation to colder and harsher conditions. (2) Allocation to storage may respond plastically to temperature, independent of sink capacity for growth. If (2), colder temperatures may trigger changes in gene expression that prioritize storage over growth. Such a response could be selected for if cold temperatures are indicative of periods of poor carbon gain and/or greater risk of carbon loss, such as tissue loss or damage (Grace et al., 2002). In both cases, increasing storage could protect against starvation but exacerbate carbon limitation to growth.

Several lines of evidence support the view that treeline trees actively maintain storage and suppress growth. In defoliated treeline trees, NSC stores were partially restored by the end of the growing season even though growth was reduced (Li et al., 2002). This situation does not support direct sink limitation to growth if NSC is excess carbon: growth should be unaffected since NSC was nonzero and temperature was unchanged. Instead it seems that storage was given priority over growth. Similarly, branch elongation and leaf length increased at the treeline when trees were debudded or pruned (Li et al., 2002; Susiluoto et al., 2007) and when deciduous trees were given

increased $CO_2$ (Handa et al., 2005). In the same $CO_2$ enrichment experiment, the evergreen species did not increase growth under higher $CO_2$ availability alone, but shoot elongation was greater when defoliated under elevated $CO_2$. These results suggest (1) temperature cannot be directly limiting growth if trees can increase growth when temperature does not change, and (2) even for the evergreen species, growth in response to disturbance or damage seems to be carbon limited. Finally, maximum photosynthetic rates at treeline and lower altitudes or latitudes may not differ (Vowinckel et al., 1975; Sveinbjörnsson, 1983; Körner, 1999). If the higher NSC levels at treeline were passively accumulating, photosynthetic down-regulation should occur, reducing assimilation rates (Bansal & Germino, 2008).

Experiments investigating treeline growth limitation and tree height offer contrasting support for sink limitation. A recent study found that experimentally heating apical buds induced a significant increase in vertical and branch growth (Petit et al., 2011). In contrast, Susiluoto et al. (2010) found that fertilization caused more than a two-fold increase in height growth. Are these cases reconcilable? Conditions among years or treeline sites could be so variable that the mechanism of limitation also varies. However, both cases can be explained by carbon limitation and active growth suppression to maintain sufficient storage. In the first case, the experimental warming released the trees from a conservative, cold-induced storage pattern and the consequently released reserves became available for growth. In the case of fertilization, the increased leaf N that resulted (Susiluoto et al., 2010) could increase photosynthesis, and potentially increase growth if more carbon was then available after the reserve needs were met.

*Water stress*

The debate between carbon and sink limitation also applies to the effects of water stress on tree growth. While reduced growth during the dry season, during moderate drought, and with increasing hydraulic limitation due to increasing tree height may be due to reduced carbon uptake (Yoder et al., 1994; Ryan & Yoder, 1997), it may also be the result of direct sink inhibition (Ryan et al., 2006; Körner, 2003).

The evidence supporting sink limitation under water stress is often very similar to that used to support sink limitation at the treeline. Because of turgor-limited cell expansion, low water availability may directly affect growth more than photosynthesis, leading to a passive buildup of excess NSC within the tree (Körner, 2003). Consistent with this hypothesis, tropical tree species during the dry season have lower growth rates and higher NSC content than during the wet season (Wurth et al., 2005), droughted seedlings decrease growth while either showing no change in NSC (Sanz-Perez et al., 2009) or an increase (Galvez et al., 2011), and tall trees often have lower branch elongation rates and higher NSC concentrations than shorter trees (Sala & Hoch, 2009; Woodruff & Meinzer, 2011).

While these observations are consistent with turgor-induced sink limitation, they are also consistent with active growth suppression to avoid the increasing threat of carbon starvation (McDowell, 2011). Because photosynthesis declines with increasing water stress, carbon starvation may be a major cause of death during severe drought (Breda et al., 2006; McDowell et al., 2008). Reduced carbon uptake and storage during a drought

may also lead to greater canopy dieback the following season (Breda et al., 2006), reducing carbon uptake and storage even more, leading eventually to starvation (Breda et al., 2006; Galiano et al., 2011). Additionally, reduced carbon uptake could reduce defense allocation, reducing a tree's ability to avoid or recover from biotic attack (McDowell 2011). Increasing reserves at the onset of water stress may allow a plant to avoid these fates, but at the cost of reducing growth.

Even if carbon starvation via NSC depletion is rare, increasing storage could still be an adaptation to drought. For example, carbon starvation could occur via phloem malfunction, before all reserves are depleted (Sala et al. 2010). But increased storage in *all* plant organs could potentially lengthen the time a plant could survive on "local" carbon without long-distance transport. In which case, mortality by phloem malfunction could still select for increased storage under water stress. Furthermore, even if carbon starvation isn't a current cause of mortality, increased storage may still be adaptive. If during a population's evolution, starvation occurred, trees may have evolved to avoid this type of mortality, leaving other ways for trees to die (e.g. hydraulic failure). The adaptive nature of increased storage is supported by the divergent response of NSC content to drought between more and less drought-tolerant tree species: the more drought tolerant increase NSC content while the less tolerant show the opposite response (Regier et al., 2009; Piper, 2011).

## Conclusion

We believe that increasing carbon storage and decreasing growth is an active response, either plastic or evolutionary, to reduce the risk of starvation—not a result of sink limitation. If true, we are left with the counter-intuitive conclusion that tree populations with the highest NSC concentrations may be the most carbon limited in terms of growth; this puts into question many recent conclusions about what limits tree growth. In fact, the rejection of the carbon storage pool as purely passive leads us to conclude that we may have greatly underestimated the importance and frequency of carbon limitation to growth in trees.

However, our knowledge of NSC dynamics in trees remains limited, and much more work is needed to determine the nature of storage and whether some populations favor storage more than others. To determine if storage is active, we may need first to determine where and when growth is carbon limited. Increasing carbon uptake experimentally or relying on natural variation in carbon uptake may be the surest way of determining limitation; if growth increases with greater carbon uptake, all else being equal, the tree was carbon limited—and any storage that occurred was active. To test if certain populations store more than others, common garden experiments could be used to compare speices' or populations' NSC concentrations or changes in concentrations relative to growth rates. Furthermore, as genetic work with Arabidopsis has demonstrated the importance of negative growth regulators, measurements of DELLA protein expression in trees from different populations and under different conditions may be useful tools in understanding when growth is constrained by environmental conditions or actively reduced by the plant.

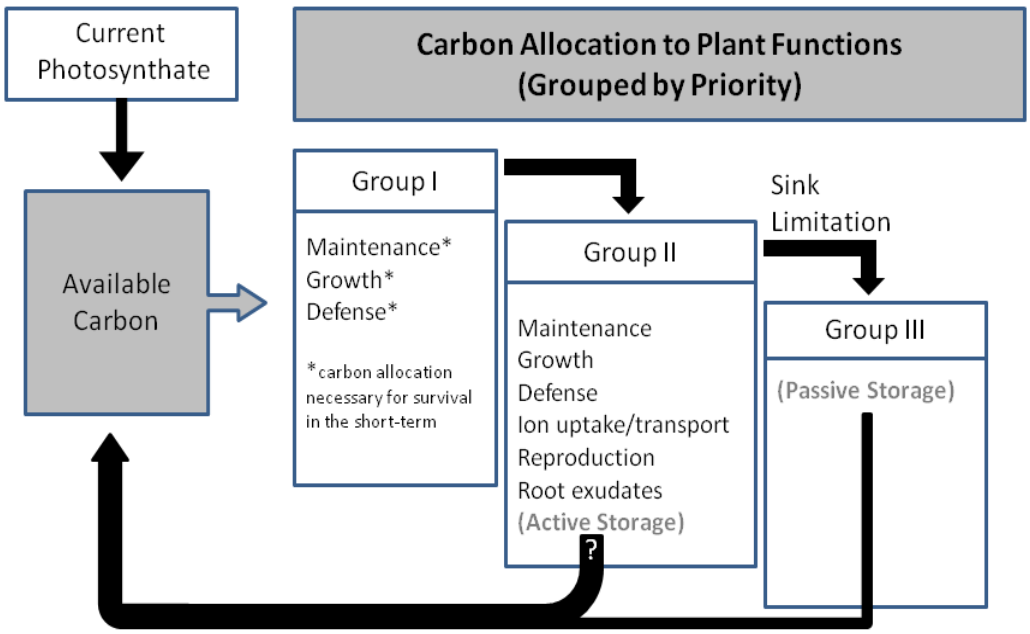



**Figure 1.1: Scheme for carbon supply and allocation to three categories of plant functions based on priority.** The order of categories indicates carbon allocation priorities, with competition for carbon occurring among processes in the same category. The order within a category does not indicate priority, however it is assumed that some priority structure does exist and can change. Group I includes the highest priority carbon needs, essential to immediate survival. Carbon starvation occurs when the amount of carbon supplied by current photosynthesis or storage to any of these processes is

insufficient to keep the plant alive. Group II includes processes which are not immediately necessary for survival, but may affect survival or fitness in the long run. For a given tree, the allocation hierarchy is determined by its life-history strategy as well as inter- and intra-annual plasticity in response to environmental cues or carbon status. Growth (or any process) can become more carbon limited by 1) a decrease in available carbon, 2) an increase in group I requirements, or 3) increased allocation to other processes (such as active storage) within group II. Group III allocation includes processes which represent overflow functions, such as passive storage. Carbon accumulates here only when processes in group II are carbon saturated.

# CHAPTER 2: CARBON LIMITATION VIA EXPERIMENTAL DEFOLIATION REDUCES GROWTH NOT STORAGE


## Abstract

There is still no consensus about how stresses such as low water availability and temperature limit tree growth. Sink limitation to growth and survival is often inferred if a given stress does not cause nonstructural carbohydrate (NSC) levels to decline along with growth. However, trees may actively maintain or increase NSC levels under moderate carbon stress, making the pattern of reduced growth and increased NSC compatible with carbon limitation. To test this possibility, we used full and half defoliation to impose severe and moderate carbon limitation on two-year old *Quercus velutina* saplings grown in a common garden. Saplings were harvested at either three weeks or four months after treatments were applied, representing short- and longer-term effects on woody growth and NSC levels.

Both defoliation treatments maintained lower total leaf area than controls throughout the experiment with no evidence of photosynthetic up-regulation and resulted in a similar woody growth reduction. While fully defoliated saplings had lower starch levels than controls in the short-term, half defoliated saplings maintained control starch levels in both the short and longer-term. In the longer-term, fully defoliated saplings had the greatest starch concentration increment (Δ% starch), allowing them to recover to near-control starch levels. Furthermore, between the two harvest dates, fully and half defoliated saplings allocated a greater proportion of new biomass to starch than did controls. The maintenance of control starch levels in half defoliated saplings indicates

that these trees actively store a substantial amount of carbon before growth is carbon saturated. In addition, the allocation shift favoring storage in defoliated saplings is consistent with the hypothesis that, as an adaptation to increasing carbon stress, trees prioritize carbon reserve formation at the expense of growth. Our results indicate that as carbon limitation increases, reduced growth is not necessarily accompanied by a decline in NSC levels. Therefore, the lack of NSC decline alone should not be used as evidence that reduced tree growth under cold or water stress is caused by sink limitation.

## Introduction

Our poor understanding of what limits tree growth and survival hinders our ability to predict how forests will respond to several global change factors, including increases in $CO_2$, temperature, insects and pathogens. We often divide mechanisms limiting growth or survival into two categories: carbon and sink limitation. This dichotomy, while perhaps overly simplistic, is very relevant to predicting how tree growth and/or mortality will respond to these future changes. Carbon limitation occurs when growth is limited by the availability of carbon. In contrast, sink limitation occurs when growth is limited by a tree's ability to use the available carbon for growth due to such factors as insufficient nutrient supply or direct environmental constraints on the rate of biosynthesis (e.g. temperature). An increase in carbon availability should only increase growth if growth is carbon-limited, not sink-limited.

Nonstructural carbohydrate (NSC) levels are often used to infer whether growth is carbon or sink limited. Consistently high NSC concentrations or reduced growth coupled with increased or static NSC concentrations in response to stress are used as indicators that growth and survival are sink limited (e.g. Korner 2003, Hoch et al. 2003, Palacio et al. 2008, Sala and Hoch 2009). Such conclusions have led to a decade of arguments and supposed evidence against carbon limitation. In particular, many experiments and studies examining the effects of water stress have concluded that growth is directly sink limited because NSC levels do not decline (Wurth et al. 2005, Sala and Hoch 2009, Sanz-Perez et al. 2009, Galvez et al. 2011, Piper 2011).

However, recent debate has arisen over whether NSC levels can be interpreted as evidence of sink limitation or not (McDowell and Sevanto 2011, Ryan 2011, Sala et al. 2012, Wiley and Helliker 2012). Specifically, it is not known whether increased NSC and decreased growth are caused by the passive buildup of carbon that cannot be used because growth is directly limited by other factors (Korner 2003), or rather, represent an active stress response to avoid carbon starvation (Smith and Stitt 2007, McDowell and Sevanto 2010, McDowell 2011). Conditions such as drought, cold temperatures, or reduced carbon uptake may all increase the chance of starvation, and a prioritization of storage over growth could enable survival over longer periods or under more severe stress.

Carbon storage dynamics, apart from their relevance to understanding how different stressors limit growth, are themselves of interest. Storage remains one of the most poorly understood and modeled carbon pools in tree growth models (LeRoux 2001).

Storage is also an important life history trait, often found to correlate with survival or recovery from disturbance (Webb 1981, Canham et al. 1999, Gleason and Ares 2004, Bréda et al. 2006, Myers and Kitajima 2007, Galiano et al. 2011). However, allocating carbon to reserves could reduce carbon available for immediate growth, defense, or reproduction. For this reason storage allocation is likely to be highly regulated and under strong selection. Trees' storage responses to disturbances are therefore important to understanding how individuals and whole forests cope with defoliation, drought, and constantly changing environmental conditions.

When comparing growth under stressful and less stressful conditions, a sink limitation to growth is often inferred if NSC concentrations are not lower in the stressful condition. However, this inference assumes that NSCs are mainly stored passively (i.e when growth is sink limited) and that trees will always maximize growth at the expense of maintaining carbon reserves. NSC and growth should both certainly decline under severe carbon limitation, as a plant must rely on stored carbon to maintain metabolism and defense for survival. But under moderate carbon limitation, if growth is more expendable than storage, storage allocation may increase relative to growth, allowing NSC levels to be maintained or even increase relative to the less stressful condition. To test this hypothesis, though, growth and storage must be monitored when carbon is known to be limiting. This requirement can be complicated because many stressors, such as drought and cold temperature, may impose both carbon and direct physical constraints on growth, and it is unclear which is the immediate limitation.

One-time defoliation is a promising candidate to test whether NSC levels can be maintained under moderate carbon limitation. Defoliation limits carbon uptake by reducing total leaf area, and when severe, it is known to decrease both growth and NSC levels (Ericsson et al. 1980, Vanderklein and Reich 1999, Li et al. 2002), which is expected under severe carbon limitation. In addition, the growth reduction observed following defoliation is correlated with the degree of canopy reduction (Ericsson et al. 1980, Salemaa and Jukola-Sulonen 1990, Reichenbacker 1996), indicative of increasing carbon limitation with declining carbon uptake capacity. Also, growth reductions following defoliation are often mitigated by higher $CO_2$ levels (Kruger et al. 1998, Lovelock et al. 1999, Matson et al. 2004, Huttunen et al. 2007), and therefore presumably greater carbon availability, which should only be possible if growth is carbon limited.

We used single defoliation treatments to test the following hypothesis: under severe carbon-limiting stress, both growth and NSC levels will decline, but under moderate stress, carbon-limited growth is not accompanied by a decline in NSC levels. We compared growth and starch concentrations of *Quercus velutina* saplings either three weeks or four months following full, half, or no defoliation (controls). The three-week response of half defoliated saplings and fully defoliated saplings relative to controls represents a test for growth and storage response under moderate and severe carbon limitation, respectively. Similar comparisons at four months allowed us to determine if the differences observed were maintained over the longer term. We then compared the change in total biomass and starch biomass from three weeks to four months among treatments to determine if there was evidence for an increase in storage allocation relative

to growth under carbon stress. Finally, we discuss whether or not our defoliation treatments actually induce carbon limitation.

## Materials and methods

*Plant material and experimental design*

Two-year old *Quercus velutina* saplings were purchased from New Jersey State Forest Nursery and kept dormant at 4 ºC until planting. In May 2010, saplings were planted 1m apart in three plots on the campus of the University of Pennsylvania (Philadelphia, PA). At planting, saplings were fertilized with a controlled release fertilizer (Osmocote® Classic 14-14-14; Scots Company LLC) and mulched. All plots were weeded and well watered by either automatic sprinklers or a drip-irrigation system. Defoliation treatments were not applied until 2011 to allow a whole growing season for recovery from transplant-shock.

*Defoliation treatments and harvest*

On June 16-17, 2011, 38 saplings (26 in plot one, 6 each in plots two and three) received one of three treatments: full (12 individuals), half (14), or no defoliation (12). For full defoliation, all leaves were removed by clipping at the top of the petiole. In half defoliation, every leaf was cut in half along the mid-vein while leaving the mid-vein intact; this form of defoliation was chosen to mimic insect herbivory more closely than complete removal of half of the leaves. For controls, no leaves were removed.

We took into account the substantial initial size variation when we assigned individuals to defoliation treatments. In June, we measured initial stem diameter with a digital caliper and leaf area with a laser area meter (CID model CI-203, Camas, WA, USA). As ground-level diameter and total leaf area were correlated ($R^2$=0.52, $p$<0.01), we took leaf area into account when assigning individuals to treatment. The smallest sapling and largest sapling were assigned to the half defoliation treatment; the rest were divided into consecutive groups of three (first three smallest, next three smallest, etc). Within each group, we randomly assigned one sapling to each of the three treatments, maintaining an even distribution of treatments among the three garden plots. After treatments were applied, we measured the total area of the leaves removed from each individual, which correlated well with our initial non-destructive estimates of total leaf area ($R^2$=0.74, $p$<0.01).

We destructively harvested half of the saplings in each treatment at mid-summer (July 9-11) and the other half at the end of the growing season (October 17-24), allowing us to examine both short-term and longer-term responses. We dug up all saplings by hand, harvesting as much of the root as possible. Because the soil had high clay content, a portion of the root system, especially the fine root fraction, was not recovered. These fine roots would not have contributed greatly, however, to total root mass.

*Growth measurements*

The defoliation treatments were expected to reduce root and stem growth, which we measured in several ways. For differences in both short- and longer-term growth, we

used woody biomass at harvest as a proxy for growth. Because treatment groups were not different in size when treatments were applied, any differences in size at the time of harvest were due to differential growth after treatment. At each harvest, aboveground woody tissue and roots were weighed after being washed, microwaved at 1000 watts for 30 seconds to denature enzymes, and oven-dried at 70ºC for one week. The effect of defoliation on total woody biomass was then analyzed separately for each harvest date using an ANCOVA after testing for homogeneity of slopes. The covariate, a measure of initial size, was derived from the first principal component (explaining 83% of variation) of a PCA using initial leaf area and initial diameters at 0 and 12 cm in height and did not differ among treatments (p=0.79 and 0.92 for July and October harvest, respectively).

We also used relative basal area increment (BAI) as a second measure of longer-term growth. The stem was marked with permanent marker at the base of the stem (0 cm) and at 12 cm above the ground (12 cm). At each mark, diameter was measured in four directions, 90º apart, before treatments were applied and again at the October harvest. For both 0 and 12 cm, we used the average diameter to calculate relative BAI in the following way:

$$(\text{Diameter}^2_{\text{harvest}} - \text{Diameter}^2_{\text{initial}})/ \text{Diameter}^2_{\text{initial}}$$

Finally, because October biomass and relative BAI include growth between treatment application and the first harvest, we wanted to ensure that any significant differences seen in the longer-term were not only the result of shorter-term growth differences. Therefore, we calculated the biomass increment ($Biomass_{\text{incr})}$ between July

and October for each treatment as the difference in average biomass between July and October.

*Carbon storage measurements*

After weighing, we sampled roots and stems—including bark, phloem, and wood—for starch content at each harvest date. Roots were sampled by pooling three 1-cm long sections, one each from the top, middle and bottom thirds of the main root axis. Stems were sampled by pooling three 2-cm long sections, one from the base, one from the base of a branch, and one from the tip of the same branch.

Starch content was then determined following the method of Baud et al (2002). Each sample was ground to powder in a ball mill, extracted twice in 80% ethanol at 80ºC for one hour to remove soluble sugars, vacuum dried, and then stored at -20ºC. Starch content was measured via enzymatic digestion to glucose using α-amylase and amyloglucosidase (A7720, A1602, Sigma-Aldrich, USA). An aliquot was removed, and its glucose content determined by digesting glucose with glucose-6-phosphate dehydrogenase and hexokinase (G8529, H4502, Sigma-Aldrich, USA) and measuring the consequent change in absorbance at 340 nm. Absorbance values were converted to moles of glucose by comparison to a standard curve derived from pure starch (S5127, Sigma-Aldrich, USA). From these values, we calculated starch content as total sample glucose equivalents, and starch content is reported as both total mass (g) and as starch level or concentration (i.e. percent of sample dry weight).

We explored the effects of defoliation on starch stores in several ways. First, we used a two-way ANOVA (main effects: treatment and harvest date) to test for differences in starch levels at each harvest date. Second, we tested for differences in the starch concentration increment, the difference in average percent starch content between October and July, as indicated by a significant interaction term between harvest date and treatment within the same ANOVA. A significant interaction between harvest date and treatment would indicate that starch levels increased at different rates in the different treatments. Third, we compared total starch mass among treatments in July and October. Total starch mass is the sum of above and belowground starch mass, each calculated as the starch concentration multiplied by the organ dry weight. The effect of defoliation on total starch mass was analyzed separately for each harvest date using an ANCOVA after testing for homogeneity of slopes. The covariate was the same as described above for biomass: the first principal component of a PCA using initial leaf area and initial diameters at 0 and 12 cm in height.

*Assessment of carbon uptake*

Because we intended defoliation treatments to reduce carbon uptake throughout the experiment, and to assess if potential carbon uptake differed between fully defoliated and half defoliated saplings following canopy refoliation, we measured total leaf area at both harvest dates. Leaf area measurements are reported as a percentage of initial leaf area, and the effects of defoliation and harvest date were examined using a two-way ANOVA.

We measured leaf-level photosynthesis to see if effects of reduced canopy size on whole plant carbon gain were negated by photosynthetic up-regulation. Leaf-level, light-saturated photosynthetic rates ($A_{sat}$) were taken periodically from the time of treatment through September using LICOR 6400 with the 6400-02B LED Light Source (LICOR Biosciences, Lincoln, NE, USA). On each date, $A_{sat}$ was measured between 800 and 1300 hours on one fully expanded leaf of two to six saplings spread among treatments, using a light level of 1200 μmol and $CO_2$ level of 400 μmol. Measurements were taken for three minutes every 10 seconds after photosynthesis had reached a plateau or after 20 minutes if no plateau was reached; an average $A_{sat}$ was calculated for each measured sapling on a given day. Vapor pressure deficit (VPD) varied strongly among sampling dates and times, and visual inspection of the data showed a strong reduction of $A_{sat}$ at VPD values > 1.75. Therefore, measurements taken at VPD > 1.75 were dropped from the analysis. Data were then divided into two groups based on date of measurement: measurements of control and half defoliated saplings taken before fully defoliated saplings completed leaf expansion (June 18-July 5) and measurements of all treatments after expansion (July 8-September 19). Then within each of these two time periods, treatment effects were analyzed using either 1) the average $A_{sat}$ for saplings that had only one measurement during that time period or 2) the maximum average $A_{sat}$ for saplings that had more than one measurement during that time period.

Finally, we measured leaf starch content as another indicator of carbon availability as leaf starch content is the balance between carbon supply and carbon demand by the leaf and the rest of the plant. Leaf starch content was measured at three

times throughout the experiment: at sunrise (AM) and sunset (PM) on June 29 and September 13/14 and at sunrise (AM) on July 9. At each collection, a single circular 1.27-cm diameter disk was punched from two leaves per individual and stored at -80ºC until processed. Samples were ground with liquid nitrogen in 2-ml centrifuge tubes, extracted in ethanol as described above, and stored at -20ºC. Starch content was then measured as described above and reported as grams of glucose equivalents per unit leaf area. Treatment effects were analyzed with separate ANOVAs for each collection date because dates differed both in the time of day when samples were collected and in treatments included (e.g. June collection did not include fully defoliated saplings, as they did not have leaves).

*Leaf Traits and Measurements*

To explore whether defoliation treatments might have imposed a sink limitation to growth, we measured several different leaf traits. We first measured leaf nitrogen content to rule out a sink limitation due to reduced nitrogen. We measured leaf $\delta^{15}N$ as it could indicate treatment differences in nitrogen dynamics, such as nitrogen uptake or nitrogen demand, caused by the loss of nitrogen and/or need to rebuild a new canopy. We also measured leaf water potential to rule out the possibility that our method of half defoliation negatively affected water balance.

Nitrogen concentration and its isotopic signature were determined for leaves collected at each harvest. Leaves removed at the time of treatment application were used to determine initial values for half and fully defoliated saplings. For most controls, a

single leaf was removed at the time of treatment to determine initial values. However, for the smallest control samplings, only half a leaf was removed to minimize leaf area reduction. Measurements were made with an elemental analyzer (Costech Analytical Technologies, CA, USA) coupled to an isotope ratio mass spectrometer (Thermo-Finnigan Delta Plus, Bremen, Germany) at the University of Pennsylvania. For each sapling, the change in percent nitrogen content and change in $\delta^{15}N$ ($\Delta\delta^{15}N$) were calculated as the difference between values at harvest time and initial values. The effects of defoliation and harvest date were determined for both variables using a two-way ANOVA.

Water potential was measured at predawn and midday on September 14, 2011 with a pressure chamber (PMS Instruments Co., Albany, OR, USA). At both times, one leaf per sapling was removed (four saplings/treatment for pre-dawn and two or three saplings/treatment for midday), immediately inserted into a plastic bag, and placed in a dark container (not longer than 45 minutes) until measured. Differences among treatments were determined using a two-way ANOVA with time of day and treatment as main effects.

*Data Analysis*

The main effects of treatment and harvest date were determined as described above for each variable (We assumed no effect of plot, as the majority of saplings were in Plot 1). When residuals were not normally distributed or the variance was correlated with the mean, data were ln-transformed (leaf area, biomass, starch mass, SLA, relative

BAI) or arcsine-transformed (starch concentration). To compare treatments within each analysis, we used either the Bonferroni correction or Tukey's HSD test to correct for multiple comparisons within the same analysis. All analyses were performed using JMP 10.0 (SAS) and graphed with Sigma Plot 12.3 (Systat). Results were considered significant for $p \leq .05$ and marginally significant for $0.05 < p \leq 0.10$.

## Results

*Carbon uptake*

Both defoliation treatments reduced canopy size in the short- (until the July harvest) and longer-term (July to October harvest), but total leaf area differences between defoliation levels were restricted to the short-term. All fully defoliated saplings produced a new canopy. New leaf growth began around two weeks following defoliation, and new leaves were not fully expanded until right before the July harvest. After refoliation, both defoliation treatments maintained lower leaf area, expressed as a percent of initial leaf area, than controls, and there was no change between harvest dates (Table 2.1; Figure 2.1). In control saplings, canopy area increased by 20% during the experiment, while canopies were reduced to 66% and 54% of initial area in half and fully defoliated trees, respectively. Therefore, for most of the short-term period, fully defoliated saplings had smaller canopies than half defoliated saplings, but between July and October, leaf area was not significantly different between these two treatments (Figure 2.1).

There was no evidence that photosynthetic up-regulation compensated for differences in canopy size. In the short-term, in fact, half defoliation reduced $A_{sat}$ from control levels ($F_{1,22}$=4.65, $p$=0.04; Table 2.2). The significantly lower leaf starch levels of half defoliated leaves in late June (across AM and PM: $F_{1,33}$=4.93, $p$=0.03; Figure 2.2) also suggest reduced carbon uptake. In the longer-term, after leaf-out was completed, $A_{sat}$ was lowest in fully defoliated saplings, but differences among treatments were not significant ($F_{2,19}$= 1.01, $p$=0.38). Similarly, early July leaf starch levels did not differ from controls for either defoliation treatment, although half defoliated saplings had marginally less leaf starch than fully defoliated saplings ($F_{2,15}$=3.20, $p$=0.07; Figure 2.2). In September, there were no differences in leaf starch among treatments ($F_{2,34}$=0.36, $p$=0.70; Figure 2.2).

*Leaf traits and sink limitation*

The trends in leaf nitrogen dynamics were not consistent with nitrogen limitation increasing with defoliation severity. Defoliation had an inconsistent but marginally significant effect on the change in leaf percent N when harvest dates were combined (Table 2.1), with half defoliated saplings having the greatest reduction and fully defoliated trees having the least. However, within each harvest date, none of the treatments differed significantly (Table 2.2). Across both harvest dates, defoliation treatment did not affect the change in $\delta^{15}N$ ($\Delta\ \delta^{15}N$; Table 2.1) but differences were apparent in $\Delta\ \delta^{15}N$ in October when half defoliated saplings underwent an enrichment in $^{15}N$ that was significantly greater than fully defoliated saplings and marginally significantly greater than controls (Table 2.2).

Finally, there was no evidence that either defoliation treatment caused water stress that might have led to a sustained sink limitation. Saplings in both defoliation treatments had significantly higher water potential than controls across both predawn and midday measurements ($F_{2,14}$=10.48, $p$<0.01; Time x treatment *NS*; Table 2.2).

*Growth*

Both defoliation treatments reduced woody growth in the short-term ($F_{2,14}$=11.08, $p$<0.01; Figure 2.3). In July, total dry weight of controls was significantly greater than half and fully defoliated saplings, indicating they had grown more since treatments were applied.

In the longer-term (October), multiple growth measurements indicate that control saplings maintained higher growth rates than saplings in both defoliation treatments. First, controls still had significantly greater mass than fully defoliated saplings and marginally significantly greater than half defoliated saplings ($F_{2,16}$=4.73, $p$=0.02; Figure 2.3). Second, relative BAI at both 0 and 12 cm height was also greater for control saplings than for defoliated saplings ($F_{2,17}$=4.60, $p$=0.03 and $F_{2,17}$=3.03, $p$=0.07, respectively; Table 2.2). Relative $BAI_{0\ cm}$ was greatest in controls, which differed significantly from that of half defoliated saplings and marginally from that of fully defoliated saplings. Relative $BAI_{12\ cm}$ was also highest for controls, but this difference was only marginally significant relative to fully defoliated saplings. Third, the biomass increment ($Biomass_{incr}$) between harvest dates for controls was approximately twice that of either defoliation treatment (Figure 2.5), indicating that defoliation had in fact reduced

growth in the longer-term, as well as in the short-term. Growth did not significantly differ between half and full defoliated saplings by any of these measures, although biomass and biomass increment tended to be greater in half defoliated saplings.

*Storage*

We predicted that only severe carbon limitation would reduce starch levels. The short-term response of storage levels was consistent with this prediction, but the response was slightly altered in the longer-term. In July, while fully defoliated saplings had significantly lower above and belowground starch levels than both control and half defoliated saplings, half defoliated saplings did not differ significantly from controls (Table 2.1; Figure 2.4). In October, there were still no significant differences in above or belowground starch levels between control and half defoliation treatments (Table 2.1; Figure 2.4). Unexpectedly, while fully defoliated saplings tended to have lower belowground starch levels than the other treatments, differences among treatments were not significant. In fact, fully defoliated saplings had aboveground starch levels identical to controls (Figure 2.4).

Total (above and belowground) starch mass, adjusted for initial sapling size, declined with increasing defoliation severity at both harvest dates (July: $F_{2,14}$=11.99, $p$<0.01; Oct: $F_{2,16}$=4.72, $p$=0.03; Appendix Figure A2.1). In July, fully defoliated saplings had significantly lower total starch mass than saplings of the other two treatments. In October, fully defoliated saplings still had significantly lower starch mass

than controls. Half defoliated saplings had starch mass intermediate between fully defoliated and control saplings, but they not differ significantly from either group.

Finally, as an estimate of storage allocation over the longer-term, starch concentration increment (change in percent starch between harvests) increased significantly with defoliation severity (Figure 2.4), suggestive of greater starch allocation. This is indicated by the significant and marginally significant interactions between treatment and harvest date for above and belowground, respectively (Table 2.1). Linear contrasts revealed that the interaction was primarily due to the greater increase in starch levels by fully defoliated trees. Fully defoliated trees had a significantly greater increase in starch content relative to controls aboveground (linear contrast *SS* = 98% of interaction *SS*; $p<0.01$) and trended in the same way belowground (*SS* = 91% of interaction *SS*; $p=0.03$), although not significantly after Bonferroni correction. Fully defoliated saplings had a greater, but not significant, increase in starch content than half defoliated trees ($p=0.07$ and 0.08 for above and belowground, respectively). Half defoliated saplings and controls did not differ significantly in starch concentration increment ($p>0.10$).

## Discussion

*Defoliation likely caused a carbon limitation to growth*

We used defoliation to determine how NSC levels and storage allocation change as growth becomes increasingly carbon limited. As expected, defoliation reduced woody growth by several measures in both the short- and longer-term. To demonstrate that this

growth reduction was due to carbon limitation, the following must be true: (1) carbon uptake was reduced and (2) growth reduction was due to reduced carbon availability, not enhanced sink limitation. Defoliation reduced whole plant carbon uptake over both the short- and longer-term. In the short-term, carbon uptake declined with increasing defoliation severity, as fully defoliated trees had no leaves for at least two weeks of this period. In the longer-term, both defoliation treatments had similar total leaf area. Leaf starch and $A_{sat}$ measurements throughout the experiment indicate that there was no leaf-level photosynthetic up-regulation, as is often observed (Hoogesteger & Karlsson 1992, Vanderklein & Reich 1999, Pinkard et al. 2007, Eyles et al. 2011), to cancel out the effects of reduced canopy area. On the contrary, $A_{sat}$ and leaf starch in half defoliated saplings were initially even lower than in controls, which suggests an even greater reduction in total carbon uptake. This reduction may be an effect of removing a portion of leaves instead of whole leaves, which we did to mimic herbivory more realistically. Effects of herbivory and/or mechanical damage on photosynthesis may expand, often transitively, beyond the area immediately affected (Zangerl et al. 2002, Delancey et al. 2008).

We found no evidence that defoliation reduced woody growth due to nitrogen loss or water stress. While repeated defoliation has been argued to impose N limitation (Tuomi et al. 1990), we do not think this is likely in our experiment. First, saplings were well fertilized and only experienced a single defoliation. Second, at each harvest date, the change in leaf N did not differ significantly among treatments. Furthermore, the change in nitrogen content with increasing defoliation severity was not consistent with

increasing nitrogen limitation, as fully defoliated trees had the smallest reduction in leaf N overall. This pattern does not seem to be a leaf age effect as fully defoliated leaves in October (~3.5 months old) did not have significantly lower leaf N than control leaves in July (~2.5-3 months old). In addition, complete defoliation increased SLA (data not shown), which is usually observed to increase with fertilization and decrease with nutrient stress (VanArendonk et al. 1997, Knops and Reinhart 2000, Calfapietra et al. 2005). Defoliated plants likewise did not suffer greater water stress, as leaf water potential was actually higher in defoliated saplings than in controls.

*Is defoliation a sink limitation?*

We did not find evidence that defoliation imposed a direct sink limitation on growth by nitrogen limitation or water stress (due to tissue damage), but sink limitation could also be imposed internally (e.g. plant architecture or phenology). Architectural constraints such as determinate growth patterns or the number of preformed buds may constrain growth and the ability to overcompensate following browsing or defoliation (Millard et al. 2001), but we did not remove buds in this experiment and so architectural constraints likely do not explain the woody growth reduction.

Defoliation can alter plant phenology (e.g. flowering time, budburst; Kolb et al. 1992, Freeman et al. 2003), and this might alter the potential for growth. For example, canopy refoliation may delay other aspects of growth or reproduction (Freeman et al. 2003). There are several reasons why this does not likely explain our results. First, only fully defoliated saplings grew more new leaf area than controls; therefore leaf growth cannot explain the growth decline in half defoliated saplings. Second, leaf expansion was

largely completed by the first harvest, so the difference between controls and half defoliated saplings in relative allocation between harvest dates cannot be explained by leaf growth phenology. Third, if refoliation was responsible for the initial reduced growth of fully defoliated saplings, it was probably due to competition for carbohydrates (Willaume and Páges 2006, 2011), and is therefore a carbon limitation.

Finally, defoliation may alter hormonal levels or other growth regulators that could potentially impose sink limitations on growth by truncating the period of growth or by simply reducing maximum potential growth rates. However, hormonally induced growth reductions are not necessarily sink limitations. For example, auxin levels and translocation from leaves affect root growth (Reed et al. 1998, Fu and Harberd 2003) and are likely reduced under defoliation (Willaume and Páges 2006), but they are in turn affected by a plant's carbon status (Lilley et al. 2012). Growth regulator levels may ultimately determine how much a plant grows, but if these levels are dependent on carbon availability, then growth is still carbon limited.

*Storage levels under carbon limitation*

Under moderate carbon limitation, we predicted that NSC levels would not decrease as growth declined, but as limitation became severe, NSC levels would also decline. The latter is expected when trees must rely on stored carbon for maintenance, canopy regrowth, and other processes necessary for survival. In the short-term, our results were consistent with this hypothesis. In the longer-term, both defoliation treatments matched our predictions for moderate carbon limitation. Control starch levels were still maintained in half defoliated trees, while growth continued to be reduced.

Fully defoliated saplings, whose severe carbon stress was partially relieved by canopy regrowth, also maintained lower growth rates but recovered to near control starch levels by the second harvest. Other species have also displayed a recovery of NSC levels before growth rate following defoliation or browsing (Reichenbacker et al. 1996, Palacio et al. 2008, Susiluoto et al. 2010, Palacio et al. 2012).

The maintenance of and recovery to control starch levels under carbon limiting conditions is not consistent with passive storage that accumulates only when growth is carbon saturated. Rather, our findings indicate that storage is an active sink competing with growth for carbon (Chapin et al. 1990, Lacointe et al. 2004, Silpi 2007). Assuming there was no sink limitation, defoliated saplings maintained control storage levels at the expense of growing larger. Such a pattern of reduced growth while maintaining similar NSC levels has been observed under other forms of reduced carbon availability including reductions in light levels (Canham et al.1999), shading in tree branches (Lacointe et al. 2004), and shortened daylength in *Arabidopsis* (Gibon et al. 2009).

The maintenance of similar starch levels across treatments suggests that a given NSC concentration may be a general requirement before growth can proceed, leading to simultaneous and proportional allocation to both storage and growth as seen in seedlings (Imaji and Seiwa 2010) and other plants (e.g. sugar beet, Watson et al. 1972). And while we should always be careful when extrapolating from saplings to adults, it seems logical that mature trees of at least some species may have a similar allocation strategy. As these tree acquire more carbon, they would only grow as much as would allow them to maintain some baseline level of provisioning, which could vary seasonally or with

environmental conditions. Therefore, trees that grow more must also increase total storage more, otherwise storage compound concentrations would decline. Such an allocation scheme seems reasonable as a bigger plant would likely require more total stored carbon during periods when photosynthesis cannot meet the immediate carbon demands for survival. By ensuring that new growth does not dilute storage levels, increasing size would not necessarily increase the risk of starvation, should some disturbance occur. Of course, this storage requirement would be revoked under extreme cases (e.g. severe defoliation) when growth may increase in priority above storage because it becomes necessary for survival.

Most of our results are consistent with previous defoliation and browsing experiments, though our interpretations differ. Heavy defoliation or browsing often leads to a reduction in both growth and NSC levels (Ericsson et al.1980, Langstrom et al. 1990, Vanderklein and Reich 1999, Li et al. 2002), but NSC levels recover before growth (Reichenbacker et al. 1996, Palacio et al. 2008, Susiluoto et al. 2010). Repeated defoliation and browsing can also cause reduced growth and similar or even increased NSC levels (Van der Heyden and Stock 1995, Palacio et al. 2008, Palacio et al. 2012). In many of these cases, the initial NSC decline is seen as evidence of carbon limitation, but the recovery of NSC levels is often interpreted as evidence of sink limitation to growth, with potential causes ranging from nutrient limitation to removal of apical meristems. However, all of these cases are also consistent with carbon limited growth as the result of reduced sink priority relative to storage.

*Storage allocation under carbon limitation*

Our findings are consistent with the idea that storage can be prioritized over growth, especially under carbon-limiting conditions. In the longer-term, defoliated saplings maintained control starch levels, but this does not necessarily imply that they allocated relatively more incoming carbon to storage than control saplings. Even the greater starch concentration increment exhibited by fully defoliated saplings may not be due to differential allocation (see Appendix for details). To determine if defoliated saplings did allocate more carbon to storage than growth between harvest dates, we considered how final starch concentration is related to initial starch concentrations and to additional biomass allocation. October and July starch concentrations can be related in the following way:

$$Conc_{oct} = Conc_{july}*(Biomass_{july}/Biomass_{oct}) + Conc_{incr}*(Biomass_{incr}/Biomass_{oct})$$

where $biomass_{oct} = biomass_{july} + biomass_{incr}$

In this formulation, October starch concentration ($Conc_{oct}$) is the weighted average of the starch concentration in July ($Conc_{july}$) plus the starch concentration of the added biomass, or biomass increment, between July and October ($Conc_{incr}$). Each concentration is weighted by the relative contribution of total July or total increment biomass to the October total biomass ($Biomass_{july}/Biomass_{oct}$ and $Biomass_{incr}/Biomass_{oct}$). $Biomass_{incr}$

and $Conc_{incr}$ represent what is added and, therefore, reflect the net allocation of carbon to storage versus structural growth pools. Compared to controls, if fully defoliated saplings went from significantly lower to statistically indistinguishable starch levels (i.e. $Conc_{oct}$ remained the same while $Conc_{july}$ declined), then fully defoliated saplings must have either (1) allocated proportionally more to storage than controls (higher $Conc_{incr}$), indicating a shift in allocation between growth and storage or (2) have added relatively more new biomass than controls with the same starch concentration (greater $Biomass_{incr}/Biomass_{oct}$ and conversely lower $Biomass_{july}/Biomass_{oct}$), representing a difference in the relative growth rate only.

Our calculations support scenario (1) because defoliation increased the starch proportion of total biomass increment from July to October (higher $Conc_{incr}$; Figure 5). Since $Biomass_{incr}/Biomass_{oct}$ was actually lower for defoliated trees (Supplementary Figure S2), scenario (2) is unlikely. Even though control saplings accumulated more total starch mass between harvest dates, starch concentration increment increased more for fully defoliated saplings because the *relative* amount of biomass allocated to storage versus growth was greater in this treatment.

Increased storage allocation may be a response to defoliation-induced carbon limitation or carbon limitation in general. Following defoliation, the chance of starvation may be increased by reduced carbon uptake, depleted carbon stores, and/or increased risk of further defoliation (if defoliation is indicative of high defoliator densities or increased likelihood of damage). It has been argued that plants should store more when the risk of carbon starvation increases, and this may only be possible by forgoing potential growth

(Chapin et al. 1990, Gibon et al. 2009, Wiley and Helliker 2012). Storage—particularly belowground—may always be a high-priority carbon sink for species like black oak that can resprout from the root collar. Maintaining high NSC concentrations at the expense of growth may ensure that these trees can survive by resprouting or refoliating after drought, fire, or gypsy moth outbreaks, even if aboveground mortality is unrelated to carbon status.

*Half versus full defoliation*

By the October harvest, half defoliated saplings had not grown or stored significantly more than fully defoliated saplings, which was expected if full defoliation imposed a greater carbon limitation. The reason we did not detect a difference could be due to (1) small sample sizes or (2) no actual difference in either growth or storage. If there was no difference in growth or October starch levels, then fully defoliated saplings—which started with significantly lower starch levels in July—must have decreased carbon allocation to some other pool (i.e. root exudates or respiration) and/or increased carbon gain relative to half defoliated saplings.

Fully defoliated saplings may have had an opportunity to increase carbon gain relative to half defoliated saplings right before the October harvest, due to differences in the timing of canopy senescence. While we did not measure leaf senescence, we did observe some at the time of the October harvest, particularly in half defoliated saplings. Differences in canopy senescence between half and fully defoliated saplings are supported by the October leaf $\delta^{15}N$ data. $^{15}N$ enrichment has been observed in senescing

leaves (Nasholm 1994, Keskitalo et al. 2005), and half defoliated leaves underwent a significantly greater increase in $\delta^{15}N$ than fully defoliated leaves in October. Furthermore, we found that the change in $\delta^{15}N$ between treatment application and October harvest ($\Delta\delta^{15}N$ correlated significantly with October starch concentration (weighted average of above+belowground) when all treatments were combined ($R^2$=0.27; $p$=0.02). One explanation for this correlation is that the timing of senescence might be modified by current carbon reserve status, allowing poorly provisioned trees (e.g. fully defoliated) to slightly delay senescence to make up for lost carbon.

*NSC levels and growth limitation*

Our data suggest that defoliation treatments caused a carbon limitation to woody growth. Our results therefore demonstrate that a lack of NSC decline can be consistent with carbon limitation. Carbon limitation has been assumed to reduce NSC levels, due to the expectation that carbon-limited trees will either use already stored carbon for growth and/or will not shunt any incoming carbon to storage. However, for this to be true, storage must mostly be passive and always have a lower allocation priority than growth. Our results indicate that neither is the case under moderate carbon limitation imposed by defoliation. In fact, instead of allocating relatively *less* carbon to storage when carbon was limited, saplings actually allocated *more*. The mechanisms underlying the observed allocation shift are not known, but it has been suggested that low carbon availability per se may trigger enhanced expression of negative growth regulators, leaving more carbon

available for storage (Smith and Stitt 2007, McDowell 2011). In any case, we should not assume that the absence of NSC level decline is indicative of carbon-saturated growth.

One could argue that defoliation did not impose a carbon limitation in this study. If defoliation instead caused a sink limitation to woody growth, it was not due to direct physical limitation from water stress or reduced nitrogen. Instead, any sink limitation would be plant-mediated, perhaps by a hormonal response to defoliation. In such a case, trees may down-regulate growth to a degree that is completely independent of carbon availability. Such a growth reduction is an active response, though, and the concurrent increase in relative carbon storage may still be regarded as the prioritization of storage over growth and part of a tree's recovery strategy.

**Table 2.1: Two-way ANOVA results for analyses with harvest date and treatment as main effects**

| | Defoliation | | | Harvest Date | | | Defol x Harvest Date | | |
|---|---|---|---|---|---|---|---|---|---|
| Response Variable | *F* | *df* | *P* | *F* | *df* | *P* | *F* | *df* | *P* |
| Leaf Area | 7.96 | 2,32 | **0.002** | 0.001 | 1,32 | 0.975 | 0.49 | 2,32 | 0.615 |
| Δ Leaf Percent N | 3.24 | 2,32 | *.052* | 1.81 | 1,32 | 0.189 | 1.03 | 2,32 | 0.369 |
| $\Delta\delta^{15}N$ | 4.53 | 2,32 | 0.212 | 0.59 | 1,32 | 0.447 | 6.43 | 2,32 | **0.005** |
| Aboveground Starch Level | 8.38 | 2,32 | **0.001** | 56.76 | 1,32 | **<0.001** | 4.48 | 2,32 | **0.019** |
| Belowground Starch Level | 13.77 | 2,32 | **<0.001** | 31.46 | 1,32 | **<0.001** | 3.02 | 2,32 | *0.063* |

Significant results are bolded; marginally significant results are italicized.

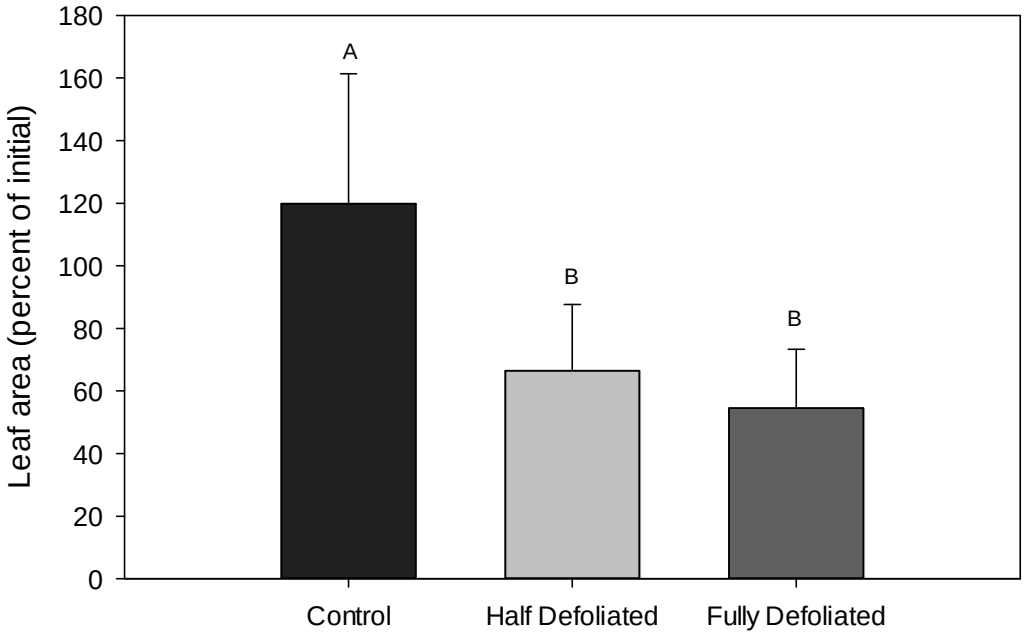



**Figure 2.1**: **Leaf area at harvest as a percent of initial leaf area**. Values represent back-transformed treatment averages pooled across both harvest dates (treatment x harvest date *NS*). Error bars represent 95% confidence limits, and different letters represent significantly different values according to Tukey's HSD.

**Table 2.2**: **Treatment averages for light-saturated photosynthetic rates, change in leaf percent nitrogen, change in leaf $\delta^{15}N$, leaf water potential, and relative BAI**

| | Control | Half Defoliated | Fully Defoliated |
|---|---|---|---|
| $A_{sat}$ ($\mu$mol $CO_2$ $m^{-2}$ $s^{-1}$) | | | |
| ***6/18-7/5*** | 11.9(.9)[a] | 9.4(.7)[b] | — |
| **7/8-9/19** | 13.8(1.4)[a] | 14.4(1.3)[a] | 11.7(1.4)[a] |
| **$\Delta$ % Nitrogen** | | | |
| ***July*** | 0.10(.07)[a] | -0.36(.23)[a] | -0.05(.08)[a] |
| ***October*** | -0.37(.14)[a] | -0.55(.25)[a] | 0.01(.13)[a] |
| **$\Delta\ \delta^{15}N$** | | | |
| ***July*** | 0.18(.16)[a] | -0.43(.56)[a] | 0.35(.47)[a] |
| ***October*** | -0.02(.30)[a*] | 1.71(.59)[b*] | -0.70(.41)[a] |
| $\Psi_{predawn}$ (MPa) | -0.5 (.1)[a] | -0.2 (.1)[b] | -0.1 (.1)[b] |
| $\Psi_{midday}$ (MPa) | -2.1(.2)[a] | -1.9(.1)[a] | -1.6(.1)[a] |
| **Rel. $BAI_{0cm}$** ($mm^2/mm^2$) | 1.07(.61-1.67)[a*] | 0.32(.06-.64)[b] | 0.43(.11-.85)[b*] |
| **Rel. $BAI_{12cm}$** ($mm^2/mm^2$) | 0.88(.20-2.21)[a*] | 0.28(-.07-.89)[ab] | 0.17(-.16-.82)[b*] |

Average light-saturated photosynthetic rates ($A_{sat}$) are given for two time periods: before and after new leaf expansion of fully defoliated saplings. Changes in leaf percent N and in leaf $\delta^{15}N$ are given for both harvest dates relative to pre-treatment values. Leaf water potential ($\Psi$) measurements were taken at both predawn and midday on September 14. Relative basal area increments were measured at both 0 and 12 cm above the ground. Standard errors or 95% confidence intervals are shown in parentheses following averages. Different letters represent significant differences among treatments, and different letters each with * represent marginally significant differences. Significant differences were determined by Tukey's HSD except for $\Delta\ \delta^{15}N$ and $\Delta$ %N, which were determined using the Bonferroni correction (# comparisons = 6).

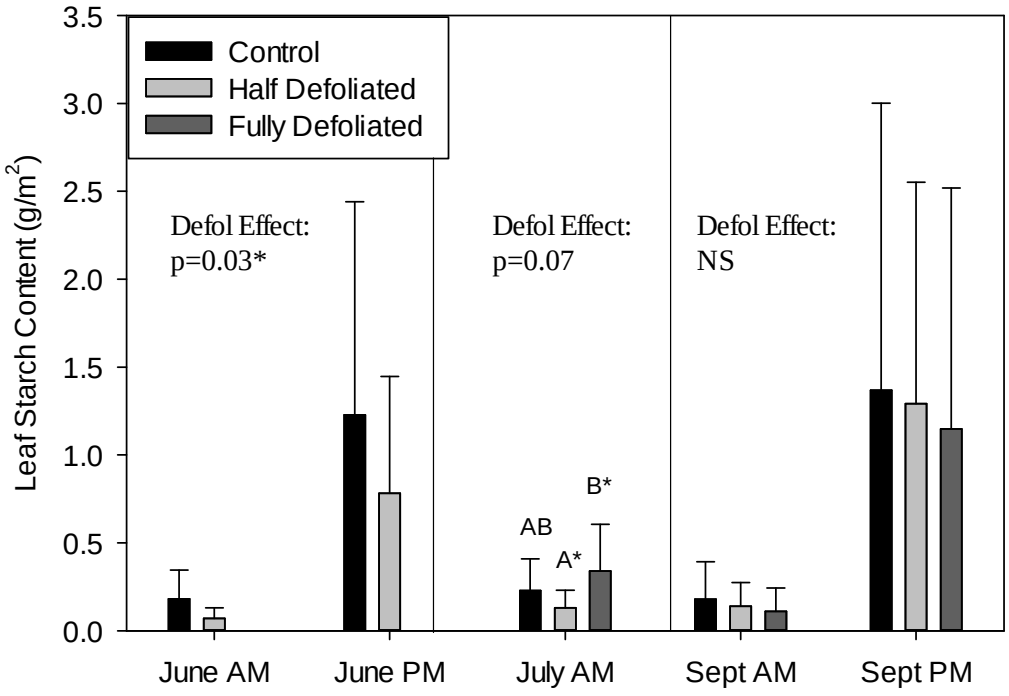



**Figure 2.2**: **Leaf starch content at three separate dates.** Error bars are 95% confidence intervals. Defoliation effects are from the separate ANOVAS performed for each date. Marginally significant differences according to Tukey's HSD are indicated by different letters with asterisks (only in July). In June, across AM/PM, half defoliated trees had significantly lower starch levels, while there were no differences in September.

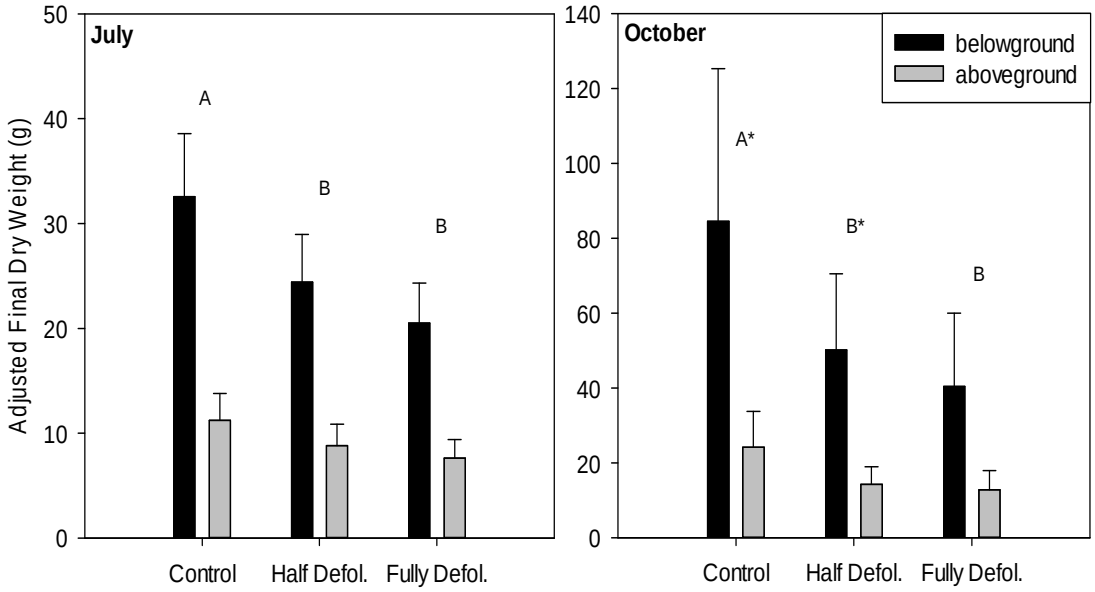
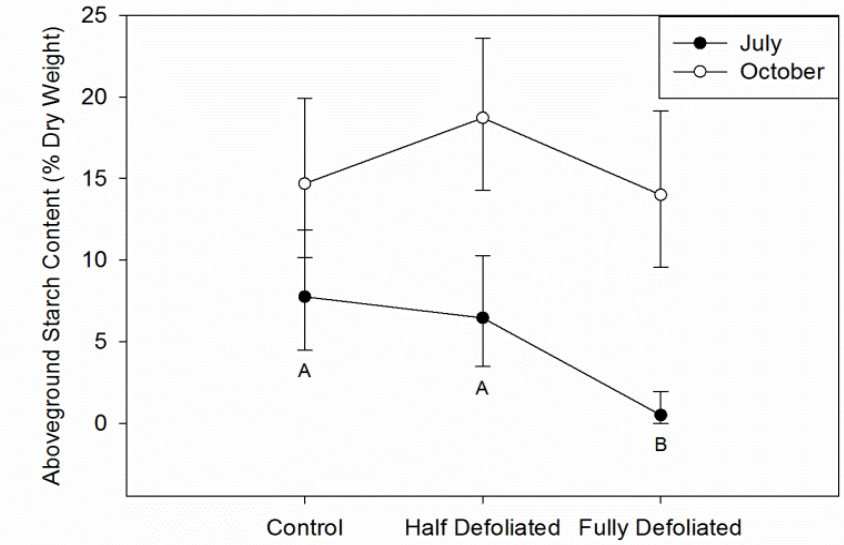
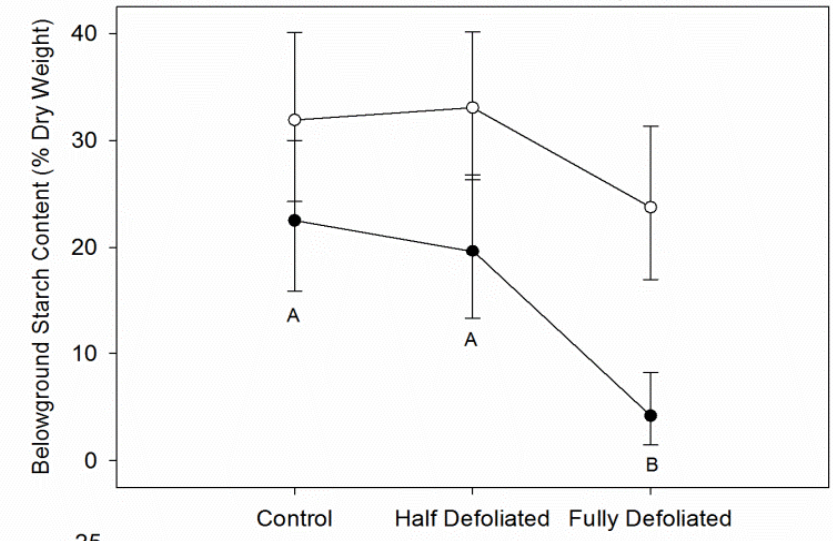



**Figure 2.3**: **Adjusted averages for final dry weight of above and belowground woody tissue.** Values are adjusted averages at harvest from the ANCOVA (Principal Component representing initial size used as covariate). Differences among treatments reflect differential growth following defoliation, as sapling size did not differ before treatment application. Error bars represent 95% confidence intervals. Different letters represent Tukey's HSD between treatments for total (above +belowground) dry weight; different letters each with * represent marginally significant differences.


Aboveground Starch Content (% Dry Weight)
25
20
15
10
5
0
July
October
A
A
B
Control
Half Defoliated
Fully Defoliated


Belowground Starch Content (% Dry Weight)
40
30
20
10
0
A
A
B
Control
Half Defoliated
Fully Defoliated

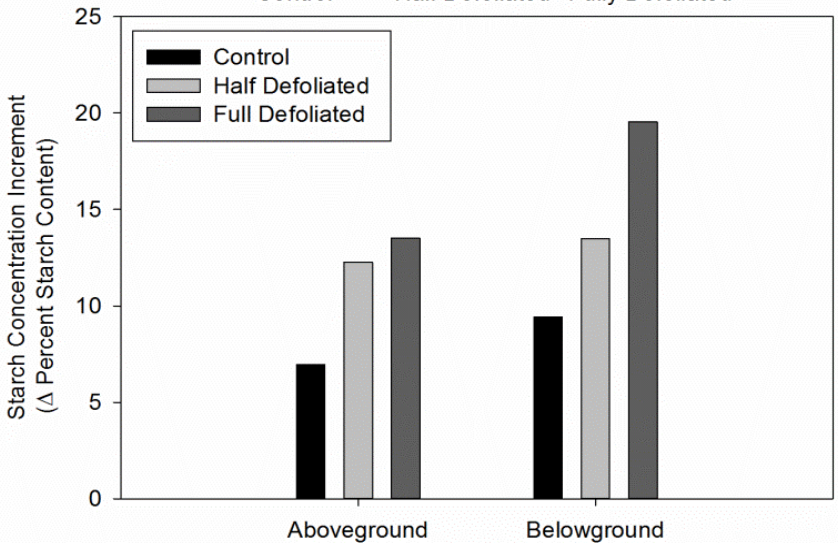

Starch Concentration Increment
(Δ Percent Starch Content)
25
20
15
10
5
0
Control
Half Defoliated
Full Defoliated
Aboveground
Belowground

**Figure 2.4**: **Starch content of above and belowground woody tissue at July and October Harvests**. Error bars represent 95% confidence intervals. Different letters represent significant differences using Bonferroni correction (# comparisons = 9) for July averages; there were no differences among treatments for October. Starch concentration increment, a measure of allocation to storage, is the difference between back-transformed October and July average starch levels. The effect of defoliation on starch increment was determined by the interaction of treatment and harvest date. The significant (aboveground) and marginally significant (belowground) interaction between treatment and harvest date is due primarily to the difference between controls and fully defoliated saplings. Fully defoliated saplings had the largest starch increments, increasing from significantly lower starch levels in July to statistically indistinguishable levels in October.

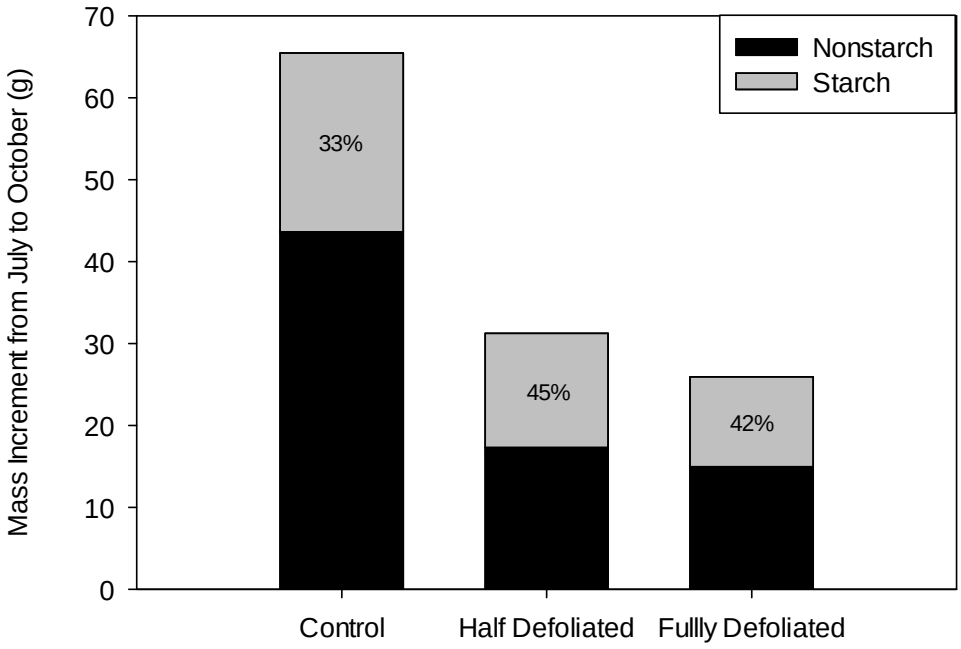



**Figure 2.5**: **Total woody mass increment (*Biomass*$_{\text{incr}}$ ) between July and October harvests, divided into starch and nonstarch components.** The average total mass and starch mass increments were calculated as the difference between the adjusted averages from July and October (adjusted by covariate of size as described in methods). Nonstarch mass increment was calculated as the difference between total and starch mass increment. Numbers represent the proportion of total mass increment due to starch, or *Conc*$_{\text{incr}}$. While defoliation tends to decrease *Biomass*$_{\text{incr}}$, it tends to increase the proportion of increment due to starch.

# Chapter 3: Carbon Availability is the Main Driver of Seasonal Dynamics in Non-Structural Carbohydrates and Growth in Adult Oak Trees


## Abstract

Nonstructural carbohydrates (NSC) are known to vary seasonally and with changes in growing condition. However, NSC variation among trees growing in similar conditions is rarely explored, and the relationship between storage and growth remains poorly understood. Using the variation among trees, we investigated the relationship between seasonal NSC dynamics and basal area increment (BAI) in an overstory oak species for two years. We introduce a simple metric, Residual BAI, to compare growth among trees across a range of diameters.

Post-leafout growth was positively correlated with NSC accumulation among individual trees. Pre-leafout growth and NSC minima were positively correlated with the previous fall's NSC levels. NSC levels did not differ between years, but post-leafout growth was greater in 2011 than in 2010, when a summer drought occurred. The relationship between growth and storage suggests that carbon availability was the main source of variation in growth among trees and that NSC accumulation was not the result of carbon-saturated growth. Furthermore, growth appears to be more expendable than storage, perhaps to ensure sufficient carbon supply for early spring growth the following year. In general, the pattern of reduced growth and static NSC levels may be more indicative of allocation priorities than of sink limited growth.

## Introduction

The carbon storage pool in trees can be mobilized to support metabolism, growth, defense, hydraulic integrity, and reproduction when current photosynthesis falls short of a plant's immediate carbon needs. Nonstructural carbohydrates (NSC) are a major component of the storage pool, and improving our understanding of NSC variation and dynamics is important for many aspects of tree and forest ecology. First, NSC levels or content are often correlated with survival and recovery from disturbance (Webb, 1981; Canham et al., 1999; Gleason & Ares, 2004; Bréda et al., 2006; Myers & Kitajima, 2007; Galiano et al., 2011), but carbon allocated to NSC storage cannot be immediately used for growth. As a result, a tradeoff between growth and storage may affect the evolution of allocation decisions and life history strategies in trees (Poorter & Kitajima, 2007; Hultine et al. 2013). Second, NSCs play an important role in the carbon cycle at the ecosystem level. NSC storage can act as buffer for net primary production (NPP), explaining the imbalance between carbon uptake and NPP either seasonally or between years (Gough et al., 2009; Richardson et al., 2013). Finally, NSC levels and dynamics have often been used as indicators of the cause of growth limitation, with high levels or low turnover/seasonal amplitude argued to occur when growth is sink limited or carbon saturated (Hoch et al., 2003; Sveinbj□rnsson et al., 2010; Woodruff et al., 2011).

NSC levels and dynamics vary in response to wide variety of factors, both genetic and environmental. NSC levels often vary seasonally (Sauter & Van Cleve, 1994, Piispanen & Saronpää, 2001, Barbaroux & Breda, 2002; Landhausser & Lieffers, 2003;

Gaucher et al., 2005; Wurth et al., 2005, Gough et al., 2009; Richardson et al., 2013; although see Hoch et al., 2003) and with relatively extreme changes in growing conditions or in response to disturbance (Bamber & Humphreys, 1965; Hoch & Korner, 2003; Galvez et al., 2011; Fajardo et al., 2012; although see Sanz-Pérez et al., 2009, Chapter 2). Under the same growing conditions, different species may differ in NSC levels (Hoch et al., 2003; Gaucher et al., 2005; Gough et al., 2009), the degree of seasonal NSC amplitude (Barbaroux & Breda, 2002; Hoch et al., 2003; Richardson et al., 2013), or even the direction of NSC response to stress (Piper, 2011). In many cases, though, we do not yet understand the reasons for this variation.

One major source of uncertainty about the causes of NSC variation is that we do not know how much allocation to the NSC pool actually affects allocation to growth. NSCs can accumulate in two different ways: when growth is carbon saturated or when carbon limited. If growth is carbon saturated (i.e. sink limited) NSCs can accumulate as excess carbon (Chapin et al., 1990); this type of NSC allocation, referred to as passive storage, does not compete with plant growth for carbon. NSC allocation can also occur at the expense of growth, or while growth is still carbon limited. This type of storage allocation is referred to as active storage or reserve formation. We know little about which type of storage process dominates in trees under any condition, but this will largely determine if NSC levels can be used as indicators of sink limitation (Korner, 2003; Sala et al., 2012; Wiley & Helliker, 2012).

While some sources of NSC variation have been well characterized, little is known about NSC variation among individual trees growing in relatively similar conditions or how this variation relates to variation in growth. When differences between species or the effects of disturbance or stress on growth NSC dynamics are explored, *average* growth and NSC levels are usually compared between seasons (e.g., spring vs. fall) or among sites or treatments (e.g. droughted vs. undroughted, treeline vs. lower elevation). Between treatments or sites, growth and NSC levels or allocation can be negatively correlated (e.g. Chantuma et al., 2009; Fajardo et al., 2012), or positively correlated (e.g. Ericsson et al., 1980; Li et al. 2002), or uncorrelated (e.g. Palacio et al., 2008; Susiluoto et al. 2010). The growth-storage relationship *within* a site or treatment, especially in mature trees, has rarely been explored (although see Palacio et al., 2012) though is just as important.

Understanding how growth and NSC allocation covary can provide insights to the causes of variation in growth and storage, the predominance of passive versus active storage, as well as the causes of growth limitation in trees. The relationship between growth and NSC allocation over the growing season will depend upon the sources of variation among trees in growth and storage. If all trees in a site have the same amount of carbon to use ($C_1$), then this level of carbon availability determines the theoretical isocline of potential growth and storage allocation options for a tree (Fig. 3.1a). Differences in allocation between growth and storage may indicate different life history strategies or allocation plasticity due to variation in growing conditions. Conversely, the decision between growth and storage may be passively determined by the degree to

which a tree's growth is sink limited. Sink limitation occurs when growth is carbon saturated, and other factors prevent the utilization of available carbon for growth. In this case, trees that are the most sink limited grow the least, and as a result, store the most. In either case, growth and storage will be negatively correlated (Fig.3.1a).

Variation in carbon availability among trees (i.e. resource availability) can also impact potential storage and growth combinations. If the carbon uptake of a single tree increases (e.g. from $C_1$ to $C_2$), all else being equal, it now moves up to a new isocline of potential growth and storage combinations (Fig. 3.1b). If there is substantial variation in carbon uptake among several trees, trees may vary most not in where along an isocline they fall, but on which isocline they reside (Fig. 3.1c). In this case, when comparing all trees, growth and storage will be positively correlated, as trees that have the greatest carbon uptake are able to grow and store more than other trees. Such a positive correlation would be expected if storage is active and the majority of trees do not experience carbon saturated growth. If there is substantial variation in carbon uptake among trees, but growth is carbon saturated (i.e. sink limited), then we should expect no correlation between growth and storage (Figure 3.1d). Instead, as carbon uptake increases and trees move from one isocline to the next, trees can only passively increase storage as they cannot increase growth.

Understanding NSC dynamics in trees requires not just a knowledge of allocation, but also of remobilization. In deciduous trees, early leaf, stem, root growth, and respiration often rely heavily on stored carbon (Gaudinski et al., 2009; Kuptz et al.,

2011), resulting in seasonal minimum NSC levels occurring early in the growing season (Barbaroux & Breda, 2002). However, NSC minima may remain relatively high (Hoch et al., 2003), and lower seasonal drawdown or turnover may be an indicator of sink-limited growth (Hoch et al., 2003; Sveinbj□rnsson et al., 2010). It is unclear how these minimum levels are related to early growth, but as with NSC allocation, the relationship between NSC remobilization and early growth may reflect a tradeoff between competing uses, variation in sink-limited early growth, and/or variation in carbon availability for both maintaining storage and growth.

To explore how variation in NSC levels, allocation, and turnover related to pre-leafout growth (2010-2012) and post-leafout growth (2010-2011), we measured wood NSC levels and radial stem growth of mature black oak trees (*Quercus velutina* Lam.). By doing so, we addressed the following questions: (1) How are growth and NSC allocation related among trees in the same site? (2) What are the major sources of variation in NSC allocation and growth among individuals and between years? (3) Does carbon availability (NSC) limit early, pre-leafout growth? (4) Are minimum NSC levels determined by carbon supply or demand?

## Material and methods

*Site and Species Description*

The study was conducted at the Rutgers University Pinelands Research Station in the Pinelands National Reserve, Burlington County, New Jersey. The soil, characterized

as a podzol, is sandy with low nutrient concentrations and pH (Tedrow, 1986; Ehrenfeld et al., 1995; Schafer et al. 2011). The climate is cool temperate, ranging from a monthly average of 0.3º C in January to 24.3º C in July (Clark et al., 2012). Annual precipitation is 1123 mm. Precipitation is generally distributed equally throughout the year, though late summer droughts are common. During our study, the 2010 growing season received less than half the rainfall received in 2011 (Song et al., 2013).

The site is characterized as upland oak/pine forest, with a mixture of chestnut oak (*Quercus prinus* L.), black oak (*Q. velutina* Lam.), scarlet oak (*Q. coccinia* Muenchh.), white oak (*Q. alba* L.), pitch pine (*Pinus rigida* Mill.), and shortleaf pine (*P. echinata* Mill.) dominating the upper canopy and *Gaylussacia* spp. and *Vaccinium* spp. dominating the understory (Skowronski et al., 2007). The forest stand was approximately 96 years old in 2010 with a maximum height of 18.8 m and average height of 8 m (Clark et al., 2010, Schafer, 2011). Overstory LAI is approximately 3.6 $m^2/m^2$ (for 2006 in Schafer et al., 2010).

Our study focused on black oak adult trees that were dominant, emergent canopies, reproductively mature. For this species, acorns mature after 2 years. Flowers emerge with leaves in late April/early May, after radial growth has begun. Pollination occurs at this time, but fertilization does not occur until the following spring (Sork et al., 1993). During their second year, acorns then develop, reaching maturity in late August to early September. In 2010, all trees flowered and produced acorns which matured in 2011.

*NEP and GPP*

Net ecosystem production (NEP; gross carbon assimilation less ecosystem respiration) and gross primary production (GPP; gross carbon assimilation), used to approximate patterns of carbon gain for black oak, were derived from 30-min average $CO_2$ eddy fluxes measured from the 19-m tall Silas Little Experimental Forest Ameriflux tower, contiguous with our site. Net ecosystem exchange (NEE) was measured by the eddy covariance method described in Clark et al. (2010), and all eddy flux data are available online (http://ornl.public.gov/amerifux). $GPP_{30\text{-}min}$ (umol $CO_2/m^2/s$) was calculated with a standard flux-partitioning procedure (Reichstein et al., 2005; Papale et al., 2006). GPP and NEP (i.e. GPP - ecosystem respiration) were then calculated by summing $GPP_{30\text{-}min}$ or $NEE_{30\text{-}min}$, respectively, over a given time period. For comparisons between years, we used the period of carbon gain from May 1 to Oct 31. For comparisons between time periods of variable length, daily NEP and daily GPP were also calculated as NEP or GPP over a given period, divided by the length of the period (days).

*Radial growth measurements and Residual BAI*

In August and September of 2009, spring-loaded dendrometer bands were installed at breast height (~1.4 m) on 20 canopy trees ranging from 12 to 36 cm in diameter. In April 2010, one additional dendrometer was installed. We began measuring

dendrometer expansion in 2010, with the exception of the band installed in April which we began measuring in July 2010. Changes in circumference were measured with a digital caliper. In 2010, measurements were made on May 5, May 26, July 7, August 29, and November 7. In 2011, measurements were made on May 5, June 2, June 16, July 11, August 25, and November 9. In 2012, one measurement was made on May 16.

For each tree, change in circumference ($\Delta C$) over a given period was converted into basal area increment (BAI) by the following equation:

$$BAI = ((C_{init} + \Delta C)/2\pi)^2 - (C_{init}/2\pi)^2$$

where $C_{init}$ is the initial circumference for the beginning of the current year. For 2010, $C_{init}$ was the circumference as measured by DBH tape. For 2011 and 2012, $C_{init}$ was calculated as the sum of the previous year's $C_{init}$ and the previous year's total $\Delta C$.

Because BAI was positively correlated with basal area (BA; see Fig. 3.2), we needed to control for this variation when comparing among years or among trees. Relative BAI ($BAI/ BA_{init}$) is sometimes used to correct for differences among size, but we found that relative BAI was also correlated with BA, but negatively so (Appendix Fig. A3.1). Therefore we used two different methods to compare BAI. First, when comparing BAI among years, after testing for homogeneity of slopes, we used ANCOVA with initial

BA as a covariate to calculate adjusted averages and detect significant differences. If time periods differed in length, we converted BAI into a growth rate: BAI divided by the length of time period in days, referred to as daily BAI. In one case, ANCOVA could not be used due to unequal slopes; instead we performed an ANOVA on the residuals from a single regression of BAI vs BA through both years' data.

The second method of comparing BAI between trees, Residual BAI, measures the deviation from expected growth, given the tree's size, and therefore removes variation due to size. First, we regressed BAI for a given time period against initial BA, and then we saved the residuals from this analysis. We then used the residual for each tree, the Residual BAI, as a comparable measure of growth (Figure 3.2). This measure indicates the direction of deviation (e.g. if negative, the tree grew less than expected) and the amount of deviation (e.g. if a large absolute value, the tree grew much more or less than expected).

*Nonstructural Carbohydrates*

To measure wood NSC levels, we collected tree cores periodically. Because we wanted to relate a tree's NSC levels to its growth, we repeatedly sampled individual trees. In 2010, we tried to capture a more detailed picture of seasonal dynamics, and so we collected samples on April 14 (before leafout, but after radial growth began), May 5 (during leafout), May 26, July 7, August 29, November 7, and December 2. Except for

December 2, we collected tree cores from approximately half of the trees to minimize damage. In 2011, we collected samples at only 4 times but with larger sample sizes: May 5 or 11, June 16, August 25, and November 2 or 9 (for sample sizes at each date, see Fig. 3.5). We collected a final sample on May 16, 2012.

Samples were taken between 1.1 and 1.2 m height. Within this region, we did not attempt to standardize the cardinal direction at each date; instead, the location of the core was picked randomly while ensuring that samples were not taken too close together. To minimize exposure of inner tree to pathogens, we sterilized the increment borer with ethanol before taking each sample and plugged the borer hole with wax after core removal. Samples were immediately stored on dry ice until we returned to the lab, where they were stored at -80ºC until processed.

From each tree core, we removed the phloem and bark from the outermost 2 cm of xylem (starch levels were found to be near zero at further depths; data not shown). Segments were microwaved at 1000 watts for 60 seconds to denature enzymes, and oven-dried at 70ºC for 96 hours.

Following the method of Baud et al (2002), we determined the starch content for all samples and soluble sugar (glucose, fructose, and sucrose) content for a subset of dates. Each sample was ground to powder in a ball mill and extracted twice in 80% ethanol at 80ºC for one hour. After each extraction, the supernatants from a sample containing the soluble sugars were removed and pooled. Pellets (containing starch) and supernatants (containing glucose, fructose, and sucrose) were vaccuum dried and then

stored at -20ºC. Starch was digested to glucose with α-amylase and amyloglucosidase. Resuspended supernatants were extracted with PVPP to remove phenolic compounds; one aliquot was removed for sucrose determination and the remainder used for glucose/fructose determination. Sucrose was digested by invertase, and fructose was converted to glucose by isomerase. Glucose content was then determined spectrophotometrically at 340 nm following digestion with glucose-6-phosphate dehydrogenase and hexokinase and comparison to standard curves using pure starch, glucose, fructose, or sucrose. Fructose and sucrose contents were calculated by subtracting actual glucose content. All NSC levels are reported as glucose equivalents percent of dry weight. Soluble sugar levels are the sum of the glucose, fructose, and sucrose percents dry weight.

We compared NSC storage in several ways. First, we simply compared NSC levels. In 2010, May 5 and May 26 NSC and starch levels did not differ ($p>0.50$), and so we combined these and refer to them as 2010 May NSC levels. Second, we calculated NSC increments, the change in NSC level from one date to another, as a measure of net storage allocation. The NSC increment over the growing season is also a measure of the seasonal amplitude in NSC levels. When considering the change in NSC from late fall to next spring, we denoted this negative increment as Winter NSC Decline. Winter NSC decline represents net remobilization of stem NSC.

*Data Analysis*

To test for differences in BAI and daily BAI between years, we used ANCOVA as described earlier. To test for differences in NSC levels and increments between years, we used paired t-tests. This approach often reduced the sample size for the analysis because it was restricted to trees for which we had measurements from both years, but it allowed us to control for substantial variation among trees. To explore the causes of variation in NSC and growth among trees within a given time period, we used linear regression analyses with each point representing an individual tree residual BAI, NSC level, NSC increment, or NSC turnover measurement.

Data transformations were used only in certain analyses if residuals were not normally distributed or were heteroscedacitic. For between year comparisons of daily BAI using ANCOVA, some dates showed patterns of heteroscedacity, and so all daily BAI values were ln-transformed to be consistent. For the regressions used to make among tree comparisons, the residual patterns did not generally violate model assumptions, and for the most part, values were left untransformed. However, because the 2010 early residual BAI values did display some heteroscedacity, we performed regressions with residual BAI values from both untransformed and ln-transformed BAI values, and both results are reported. All analyses were performed using JMP 10.0 (SAS) and graphed with Sigma Plot 12.3 (Systat).

## Results

*GPP and NEP*

From May 1 to October 31, GPP and NEP were both lower in 2010. GPP was 87 mol $CO_2/m^2$ in 2010 and 119 mol $CO_2/m^2$ in 2011. NEP was -13.6 and -16.7 mol $CO_2/m^2$ in 2010 and 2011, respectively. The lower NEP and GPP in 2010 was in part due to a late summer drought that affected most of August and September.

Average daily GPP in 2010 ranged from 62% to 91% of that in 2011 among the four periods considered (Table 3.1). Average daily NEP did not vary as consistently, and was sometimes slightly higher in 2010.

We also found that when combining both years, daily BAI was strongly and positively correlated with average daily NEP for the same time period (Fig. 3.3). Daily BAI was only weakly positively correlated with GPP ($R^2_{adj}$=0.30, p=0.094).

*Basal Area Increments Between Years*

Total BAI and post-leafout BAI were both lower in 2010 than in 2011, by 20% and 21% respectively (Total BAI: $F_{1,38}$=6.00, p=0.019; post-leafout: Fig. 3.4), but at smaller time scales, growth did not always differ between years (Table 1). Early (pre-leafout), May, September/October, and the following year's early daily BAI did not differ significantly among years. In June, daily BAI was significantly lower in 2010 than in 2011, and in July/August it was marginally significantly lower. Both these intervals contained periods of drought conditions (Song et al. unpublished).

The correlation between basal area and Daily BAI during July/August also differed between years. In 2010, BAI was positively correlated with BA ($R^2_{adj}$=0.72, p<0.001) as expected, but there was no relationship in 2011 ($R^2_{adj}$=0.0, p=0.33). In addition, the reduction in daily BAI from June to July was not a function of size in 2010. However, in 2011, there was a weak positive correlation between BA and the reduction in daily BAI from June to July (p=0.058).

*Intra- and Interannual Variation in NSC*

Starch levels varied strongly among trees, but a distinct seasonal pattern was observable in the average, minimum, and maximum values in both years (Fig. 3.5). In general, starch levels were lowest at the time of and immediately following leafout (May), and generally increased over the growing season, reaching a maximum in early November, around the time of canopy senescence. Starch levels then declined in December, as trees became dormant. This decline was due in part to a slight but significant decline in total NSC, but mostly due to a conversion of starch to glucose and fructose (Appendix, Figure A3.2). In December, total NSC level was positively correlated with soluble sugar levels ($R^2_{adj}$ =0.41, p=0.002), which was mostly due to the correlation between total NSC and sucrose ($R^2_{adj}$ =0.64, p<0.001).

While growth was lower in 2010 than in 2011, there were no significant differences in NSC levels or increments between years in May, August or November (Table 3.2). However, there were differences in how NSC was partitioned between starch and soluble sugars. 2011 starch levels were marginally higher in August and

November, while 2010 sugar levels were higher in both August and November (Table 3.2). As a consequence, starch made up a greater proportion of total NSC in 2011 than in 2010 in both August ($t=5.7$, $p=0.002$) and November ($t=2.4$, $p=0.034$).

*Post-leafout Growth and NSC Allocation*

Overall, trees that allocated more to storage tended to grow more than expected in 2011. Post-leafout residual BAI was positively correlated with both November NSC levels and NSC increment (Fig. 3.6c,d). In addition, there is no indication that this relationship was leveling off, which would be expected if trees with the highest NSC and growth levels were sink limited.

Over shorter periods of time within 2011, the starch increment was never negatively correlated with residual BAI. From both May-June and June-August, there was no significant correlation ($R^2_{adj}$ =0.03, p=0.25 and $R^2_{adj}$=0.0, p=0.66, respectively), and if there was a trend it was positive. From August to November, starch increment was positively correlated with residual BAI over this time ($R^2_{adj}$=0.44, N=11, p=0.016).

In 2010, there was a similar positive trend between post-leafout residual BAI and December NSC increment, but this relationship was not significant (Fig. 3.6b). There was also no relationship between 2010 residual BAI and December (Fig. 3.6a) or November NSC levels (data not shown). Instead, the leveling off of residual BAI as December NSC levels increase may be an indication of carbon saturated growth for trees with highest NSC levels.

Across both years, November NSC was positively correlated with May-November NSC increment ($R^2_{adj}$ =0.65, p<0.001) and with beginning May NSC levels, though not as strongly ($R^2_{adj}$ =0.16, p=0.022). NSC increment and May NSC levels were not correlated ($R^2_{adj}$ =0.0, p=0.41).

*Early Growth, May NSC Levels, and Winter NSC Decline*

Fall NSC levels in either November or December appeared to, at least in part, determine the proceeding winter's NSC decline, early growth, and May NSC levels. In both 2011 and 2012, early residual BAI was positively correlated with the previous year's NSC levels at the end of growing season (Fig. 3.7). Winter NSC decline following 2010 was positively correlated with December NSC levels ($R^2_{adj}$ =0.58, p<0.001), and winter starch decline following 2011 was similarly correlated with November starch levels ($R^2_{adj}$ =0.62, p<0.001). May 2011 NSC and 2012 starch levels also tended to correlate positively with the previous year's late NSC levels (Table 3.3).

Apart from the previous year's NSC levels, May NSC or starch levels were not consistently related to other factors considered. In 2010, May NSC was negatively, but not significantly related to early residual BAI (Table 3.3). 2010 May starch levels were slightly more correlated with early growth ($R^2_{adj}$ =0.23, p=.048). In 2011 and 2012, May NSC and starch levels were positively correlated with early residual BAI, but these correlations were driven by a single point/tree (Table 3.3). There was no relationship between May NSC or starch levels and preceding winter NSC decline (Table 3.3).

## Discussion

*Seasonal NSC and BAI Variation*

The strong seasonal variation in starch and NSC levels observed for black oak is common among deciduous species (Sauter & van Cleve, 1994; Barbaroux & Breda, 2002; Gaucher et al., 2005; but see Hoch et al., 2003) and corresponds well with our understanding of their phenology. Minimum levels in May reflect the remobilization of stored carbon for leaf, stem, and flower production, which emerge at approximately the same time as leaves in black oak. NSC levels then slowly increase to a maximum at the end of the growing season, with a greater seasonal NSC amplitude often found in ring-porous species, like oaks (Barbaroux & Breda, 2002; Newell et al., 2002; Chantuma et al., 2009). Then, as commonly seen for temperate deciduous species, starch is converted to sugars at the beginning of winter dormancy (Sauter and van Cleve, 1994, Barbaroux & Breda, 2002; Landhausser & Leiffers, 2003; Améglio et al., 2004; Richardson et al., 2013) and is associated with increasing freezing tolerance (Morin et al., 2007) and winter embolism repair (Améglio et al., 2004).

We also observed seasonal variation in radial growth rates during the growing season, but these differences may have been driven by carbon gain. NEP and GPP (weakly) were positively correlated with daily BAI when comparing monthly to bimonthly averages, and this pattern was repeated when we compared annual GPP and growth. Similar correlations between NEP and radial or woody growth have been described on annual (Ohtsuka et al. 2009, Zweifel et al., 2010), and less frequently

monthly, time scales (Zweifel et al. 2010). Cases of a one-year lag (Gough et al. 2009, Richardson et al. 2013) or no correlation (Rocha et al. 2006) have also been reported. These positive correlations suggest carbon limited growth, though we cannot easily separate effects of changing carbon uptake with phenology or changes in water availability, as they all tend to be correlated.

While the seasonal variation in NSC levels and growth that we observed here is commonly reported, there was substantial variation among trees that is seldom investigated, at least in mature trees. Below, we discuss the causes of this variation in NSC levels, NSC increments, and growth and what they indicate about growth limitation and carbon allocation in trees.

*Variation in growth and storage among trees*

Differences in carbon availability appear to be the main source of variation in growth, storage, and NSC remobilization among black oaks at our site. The positive correlation between post-leafout growth and NSC increment, though only significant in 2011, indicates that trees that stored more also grew more, most likely because they had more carbon with which to do both. Stored carbon is the carbon supply for both pre-leafout growth and net remobilization of NSC, measured as winter NSC decline. Pre-leafout growth and NSC winter decline (net remobilization) were positively correlated with carbon supply. For these processes, the carbon supply is stored carbon as approximated by the previous fall's NSC levels.

Given that carbon supply is the major cause of growth variation, then carbon availability rather than sink limitation limited radial growth in these trees, with the possible exception of growth in 2010. A corollary to this is that NSC allocation was dominated by active storage. We predicted that sink limited growth and consequent passive storage would be manifested in either (1) a negative relationship between growth and NSC increment (as trees that are more sink-limited can use even less carbon, thus shunting more to storage) or (2) no correlation between growth and NSC increment (as increases in carbon uptake will only allow storage, not carbon-saturated growth to increase). In 2011, the relationship between growth and NSC increment was positive and did not appear saturated. In 2010, though not significant or only marginally so, there was still a trend toward a positive correlation between growth and NSC allocation. But there may have been a slight saturation of growth at higher NSC *levels* (see Fig.6a), indicating some degree of sink limitation and passive storage, perhaps during the drought.

While differences in carbon availability may have been the major source of growth and storage variation over the growing season, other dynamics governing allocation (e.g. variation in sink limitation, genetic variation affecting allocation) are also likely occurring and may be more important over periods of lower net carbon uptake. Over shorter periods of time, differences between trees in carbon gain may be relatively small, and so differences in tradeoff decisions or in sink limitation may be more prominent. This could explain why positive correlations between growth and NSC increment were often not detected over shorter periods *within* the growing season. Over the whole growing season, though, the effects of smaller differences in carbon uptake

rates become more pronounced as the gap in cumulative carbon uptake increases. The reduction in GPP in 2010 may similarly explain why the positive trend of post-leafout growth versus NSC increment in 2010 was not significant, particularly if trees with highest potential carbon uptake experienced the greater photosynthetic reductions.

We found little evidence that spring minimum NSC levels were determined by sink limitation or demand. If sink limitation dictated minimum NSC levels, then we should see one of two patterns: (1) a negative relationship between early growth and early NSC levels if variation in early growth is due to variation in the degree of sink limitation or (2) no relationship if all trees were similarly sink limited but differed in carbon supply (i.e. stored carbon) and so would only differ in minimum NSC levels. While there was a weak negative correlation in 2010 and possibly no correlation in 2011 or 2012, we also saw that early growth was positively correlated with the previous year's NSC levels. The correlation between early growth and carbon supply suggests growth was not strictly sink limited. Furthermore, if minimum NSC levels were only determined by how much carbon a tree could use, then NSC winter decline (i.e. NSC remobilization) should be negatively correlated with minimum NSC, but they were uncorrelated.

Early growth and NSC levels could also be uncorrelated if there are restrictions on NSC remobilization to protect against damage or reduced photosynthetic capacity (defoliation, drought, storm damage, etc) or to ensure enough carbon was available to support acorn maturation. In this case, trees may ensure a baseline NSC level and then only use excess carbon for additional growth (Lacointe et al., 2004). Similarly, baseline

requirements may exist for storage *and* growth, and once these are met, a tree with more carbon could use the remainder to increase both growth and minimum NSC level. This could explain the slight positive correlation we sometimes observed. Increasing both growth and storage as carbon availability increases may help balance different aspects of fitness. Growth allocation can increase resource acquisition and competitive ability and is sometimes associated with defense (i.e. resistance to bark beetle attack; Christiansen, 1985). Storage allocation can increase survival (see earlier references) and defense (e.g. Matson & Waring, 1984; Goodsman et al., 2013) and can be used for reproduction (Ichie et al, 2012.)

Though carbon availability was likely the main source of variation and proximate driver of radial growth and storage allocation, we cannot determine what limited carbon availability, and ultimately, growth. Furthermore, the relationship between storage allocation and other aspects of growth may be quite different, potentially driven by variation in sink limitation. It is possible, for example, that nitrogen or phosphorus supply limits canopy growth, which to a large extent may determine carbon availability for radial growth and storage. In this case, though carbon is the proximate limitation to radial growth, nutrients may still be the ultimate limitation controlling carbon availability.

*Between Year Variation in Growth and Storage*

The reduction in BAI but not NSC levels or increment in 2010 is consistent with the drought imposing a sink limitation to growth. While we did observe positive

correlations between GPP, NEP, and growth between years, suggestive of carbon limitation, the drought could also have reduced growth capacity via reduced turgor pressure. Low water availability often reduces growth but not NSC levels, and this has been taken as evidence of sink limitation (Wurth et al. 2005, Sala and Hoch 2009, Sanz-Perez et al. 2009, Galvez et al. 2011, Piper 2011). Consistent with this interpretation, the regression of 2010 growth versus December NSC levels suggests a slight saturation of growth at higher NSC levels, indicative of carbon saturated growth.

An alternative interpretation of the reduced growth and static NSC levels is that growth is more expendable than storage. Similar reductions in growth but not NSC levels have been reported for several cases where reduced growth is almost certainly due to reduced carbon uptake such as reduced daylength (e.g. *Arabidopsis*; Gibon et al. 2009) or light (among branches; Lacointe et al. 2004). In these cases, and potentially during the 2010 drought as well, maintaining storage levels was prioritized over maintaining growth levels. The maintenance of storage may be a mechanism to protect against carbon starvation (Smith and Stitt 2007) or in the case of our oaks, to ensure sufficient carbon for the development of the acorns initiated in early 2010. In addition, late season NSC storage may be given priority over growth because of the role of NSC in cryoprotection (Tinus et al. 2000) and winter embolism repair (Ameglio et al. 2004). In our study, higher total NSC levels in December translated into higher soluble sugar levels as well, particularly higher sucrose levels, suggesting trees that stored more were more protected against damage from cold temperatures.

Late season storage may also be favored over immediate growth to ensure enough carbon to support the *next* year's growth. In this case, the 2010 reduction in growth represents a tradeoff between current late-season growth and future early-season growth where early growth has priority. The existence of this tradeoff is supported by experimental growth manipulations of aspen seedlings, in which reduced height growth correlated with greater year-end NSC concentrations and greater height growth the next year following transplantation (Landhausser et al., 2012). We too found that early growth correlated with previous fall NSC levels, which did not differ between 2010 or 2011. As a result, the following year's pre-leafout growth did not significantly differ either. Prioritizing early growth may be particularly important for ring-porous species like black oak which tend to suffer major winter cavitation and require the formation of new xylem before canopy conductance can be restored (Breda & Granier, 1996).

A third potential explanation for the reduced growth in 2010 is that growth was in fact directly limited by carbon supply and that actual storage was also lower. While total NSC levels were not significantly lower in 2010, a larger portion of NSC was in the form of sugars in both August and November. Unlike starch, soluble sugars also have non-storage functions. Higher soluble sugar content in 2010 may be the result of increased water stress during the drought. Higher NSC levels may have been required to maintain cell turgor and hydraulic integrity of the xylem (Ingram & Bartels, 1996; Woodruff & Meinzer, 2011; Sala et al., 2012), with less NSC content actually functioning as storage. The marginally lower starch levels in 2010 may therefore reflect a slightly lower carbon availability for both growth and storage in a strict sense.

Apart from the effects of drought, more subtle growth differences between years may have arisen due to acorn production. BAI generally correlated well with size, except right before acorn maturation in July/August 2011. Size-independent growth during this time may be due to a greater impact of acorn production on larger than smaller trees. Older and larger trees often allocate more carbon to reproduction in both absolute and relative amounts (Genet et. al., 2010; reviewed in Thomas, 2011). A greater allocation of carbon to acorns during this time may have left less available for radial growth in larger trees, causing BAI to decline to smaller tree levels. This scenario is consistent with larger trees experiencing a marginally greater decline in daily BAI from June to July/August than smaller trees in 2011, but not in 2010.

*NSC dynamics as indicators of growth limitation*

NSC levels have been argued to be a good measure of a tree's carbon status and as a measure of the imbalance between C supply and growth demands (Korner 2003); our data support the first claim but not the second. The positive correlation between growth and NSC levels demonstrates that trees that grew the least also stored the least, and likely had the least carbon. This positive correlation has also been observed between treatments that varied in light exposure (Veneklaas & den Ouden, 2005) or defoliation (e.g. Ericsson et al., 1990; Li et al., 2002), both cases where differences are due to carbon availability. However, we saw no evidence that increased NSC levels reflected an increased imbalance between potential sink strength and supply. The fact that trees with more NSC were likely *less* carbon limited does not mean that their growth was carbon saturated and

therefore unlikely to be stimulated by further increases in carbon availability. Carbon saturation should be accompanied by a saturating relationship between growth and NSC levels, which may have occurred in 2010, but did not in 2011.

Seasonal NSC amplitude has been suggested as a better indicator of the degree of carbon limitation (Hoch et al. 2003, Wurth et al., 2005, Sveinbj□rnsson et al., 2010). Carbon limitation is expected to be manifested in large seasonal NSC swings due to periods when large C demands require the use of stored C. Lower seasonal amplitude is expected as sink-source imbalance increases and growth becomes more sink-limited. For example, Woodruff and Meinzer (2011) found that the tallest trees had the smallest NSC amplitude and the lowest branch extension. Similarly, when comparing white spruce in different mountain ranges, Sveinbj□rnson et al. (2010) found that branch extension was lowest when branches exhibited the smallest decline in NSC content (Δ mg). In both these cases, reduced growth was concluded to be due to sink limitation from low turgor or temperature, respectively.

The positive correlation between NSC increment and growth in our study, however, suggests seasonal NSC amplitude is not a reliable indicator. Higher NSC amplitude was found in trees that grew and stored the most, and therefore in the trees that were likely the least carbon limited. Trees here with the *smallest* NSC amplitude were likely the *most* carbon limited, and their low NSC amplitude reflects less carbon with which to grow or store. The same could be argued for the trees in Woodruff and Meinzer (2011) and even for Sveinbj□rnson et al. (2010).

*Conclusion*

We investigated seasonal NSC dynamics and growth by exploring the variation among emergent, adult trees within a site. Variation in carbon availability was the main driver of differences in growth and NSC levels, allocation, and remobilization. While we cannot rule out the possibility that sink strength or nutrient availability drives carbon gain, and as a result, ultimately drives carbon use, the positive correlation between growth and NSC increment suggest that NSC accumulation in these trees is largely an active process, not the result of carbon-saturated growth. Furthermore, the maintenance of NSC levels between years that differed in GPP and radial growth may indicate that growth is more expendable than storage—particularly later in the growing season. Prioritizing storage may ensure sufficient carbon supply for early spring growth the following year. In general, the pattern of reduced growth and static NSC levels may be more indicative of carbon allocation priorities in trees than of sink limited growth.

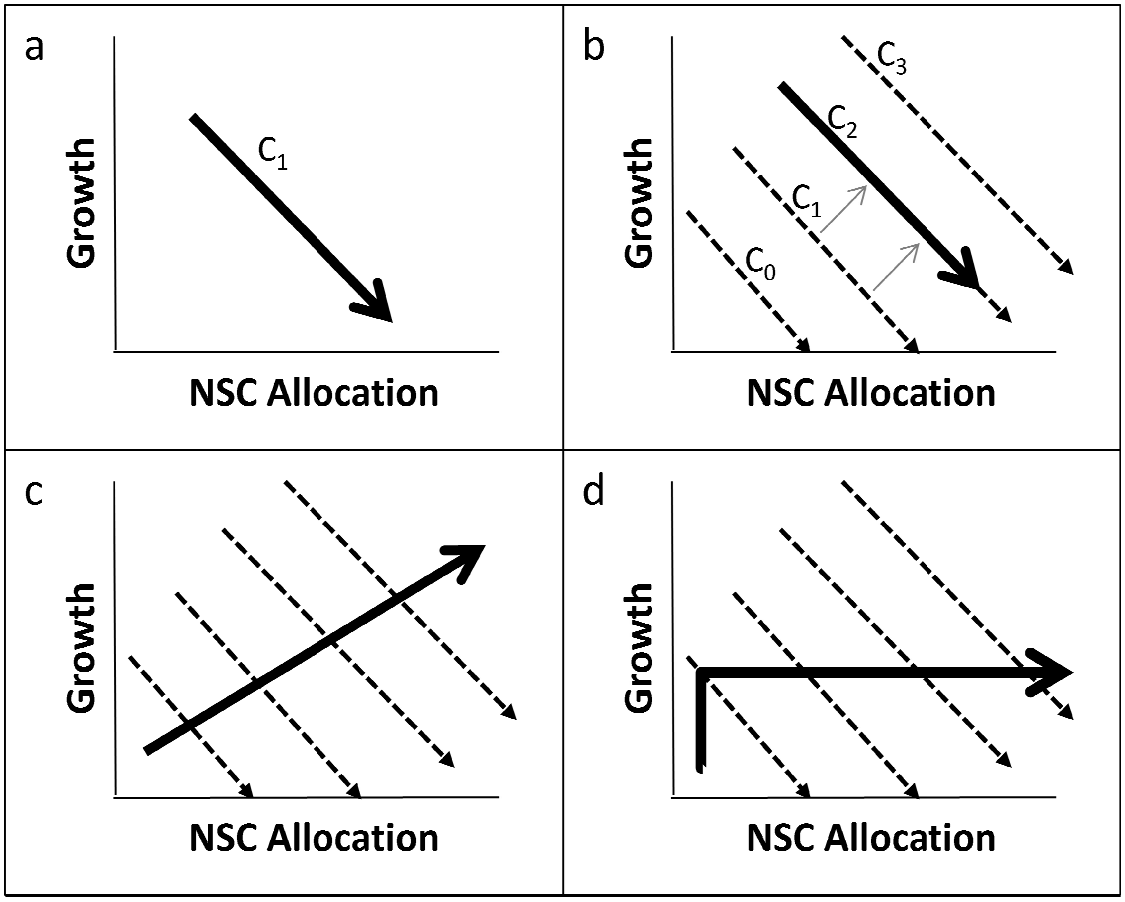



**Figure 3.1: Potential Relationships Between Growth and NSC Allocation (or Levels)**

Hypothetical growth vs. storage relationships are determined by variation in carbon availability, allocation tradeoff decisions, and degree of sink limitation. a) Growth and NSC allocation are negatively correlated. For a fixed amount of carbon ($C_1$ ), trees that grow more will have less to store, leading to a tradeoff curve. If this relationship occurs, trees vary either in their allocation strategy (either genetically fixed or plastic) or in the degree that growth is sink limited. If the variation is due to sink limitation, variation in NSC allocation is passive. b) Under increased carbon availability, trees shift up to a new resource isocline ($C_2$), with a new theoretical set of growth and NSC allocation

possibilities. c) When there is substantial variation in carbon availability (i.e. trees tend to fall on different isoclines) then growth and NSC allocation will be positively correlated if tree growth is carbon limited. In this case, variation in NSC allocation is active. d) When there is substantial variation in carbon availability but growth is carbon saturated/sink limited, NSC allocation and growth should be uncorrelated. In this case, trees that uptake more carbon can only passively store more NSC than other trees because additional growth is not possible under the current conditions.

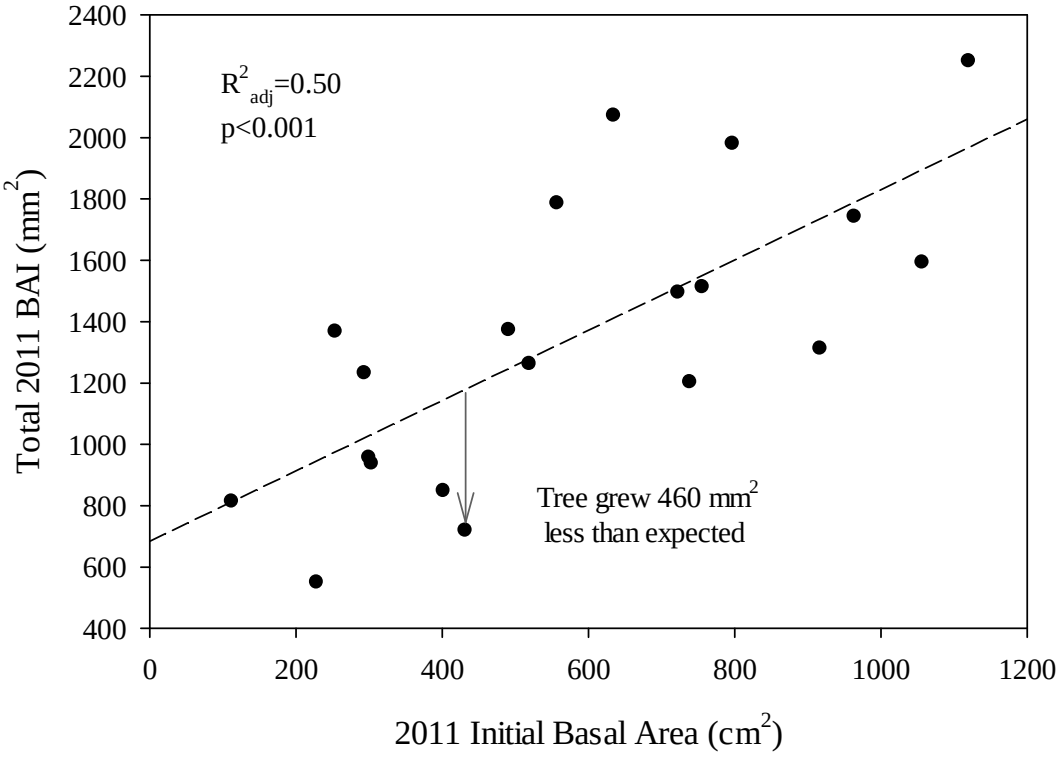



**Figure 3.2**: **Calculation of residual basal area increment**. To compare trees, we used residual BAI as a measure of radial growth, calculated as the residual from the regression of BAI vs that year's initial basal area. In the example here, a tree's residual BAI for 2011 is shown as the difference between the tree's actual BAI and its expected BAI, given its initial size. This method of comparison represents a method of comparing tree growth while controlling for initial size.

**Table 3.1**: **Average daily basal area increment (BAI), daily gross primary production (GPP), and daily net ecosystem production (NEP) for 2010 and 2011**

| Period | **2010 daily BAI** ($mm^2/d$) | **2011 daily BAI** ($mm^2/d$) | **2010 daily GPP,NEP** (mol $CO_2/m^2/d$) | **2011 daily GPP,NEP** (mol $CO_2/m^2/d$) |
|---|---|---|---|---|
| **Early** | $9.1^{a}$ | $9.8^{a}$ | | |
| **May** | $8.4^{a}$ | $8.6^{a}$ | 0.52, 0.16 | 0.66, 0.13 |
| **June** | **$7.0^{a}$** | **$9.5^{b}$** | 0.78, 0.25 | 0.85, 0.27 |
| **July/Aug**** | *$2.7^{a*}$* | *$3.5^{b*}$* | 0.52, 0.12 | 0.83, 0.11 |
| **Sept/Oct** | $1.6^{a}$ | $2.1^{a}$ | 0.23, -0.08 | 0.44, -0.03 |
| **Following Year Early** | $9.8^{a}$ | $9.0^{a}$ | | |

Daily BAI is the back-transformed adjusted or unadjusted (for July/August only) average BAI divided by the number of days in the period. Significant and marginally significant ($p<0.10$) differences are indicated by different letters and different asterisked letters, respectively. Daily GPP and NEP are the sums of GPP and NEE over the time period divided by the number of days in the period.

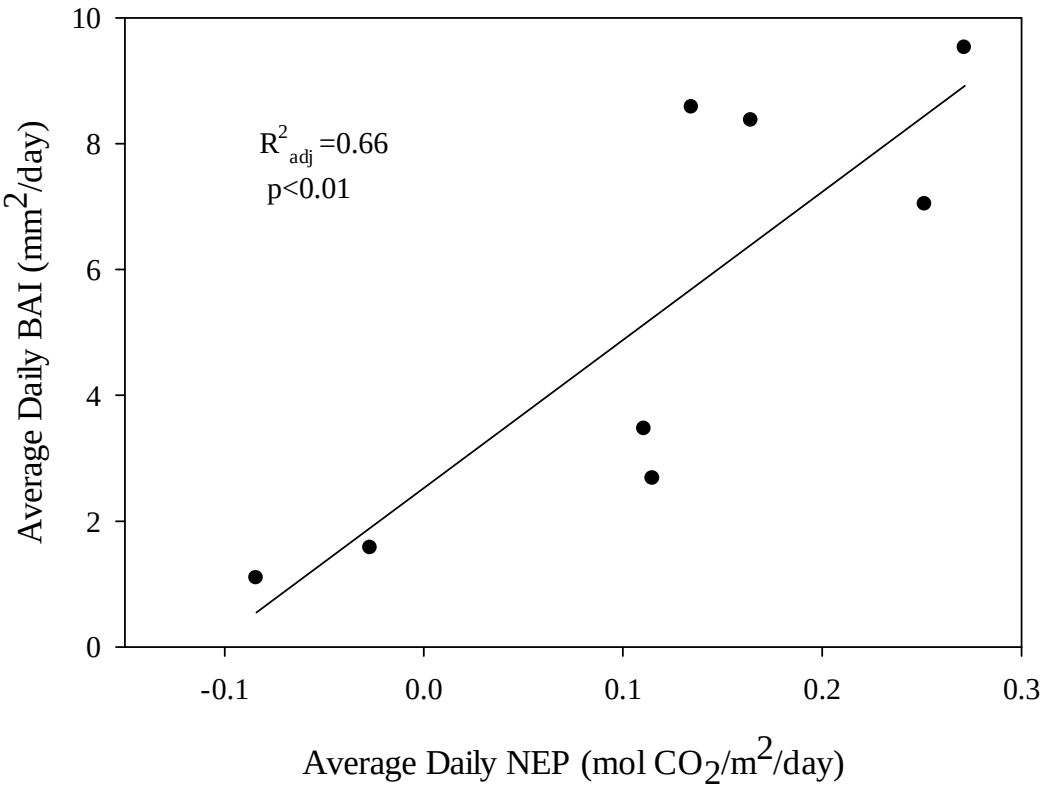



**Figure 3.3**: **Daily BAI vs Daily NEP**. Data points are for each monthly or bimonthly time period from Table 1, except for early periods (pre-leafout).

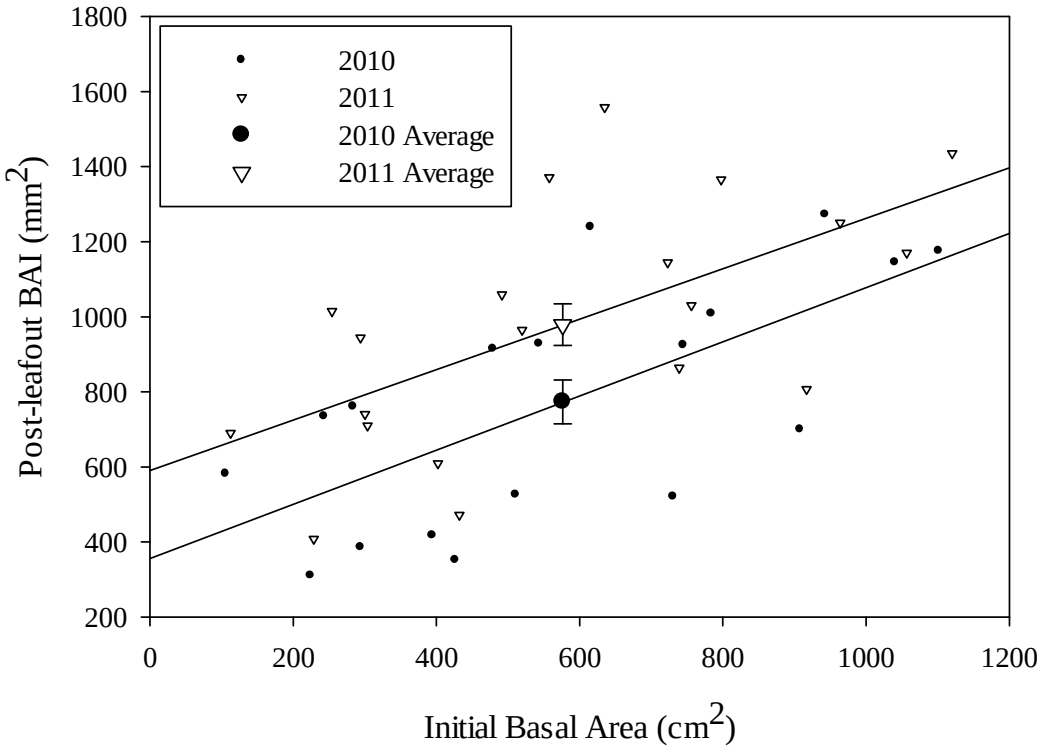



**Figure 3.4**: **2010 and 2011 post-leafout basal area increment (BAI).** Data points represent individual trees. Adjusted averages and regression lines are from the ANCOVA. Error bars are standard errors.

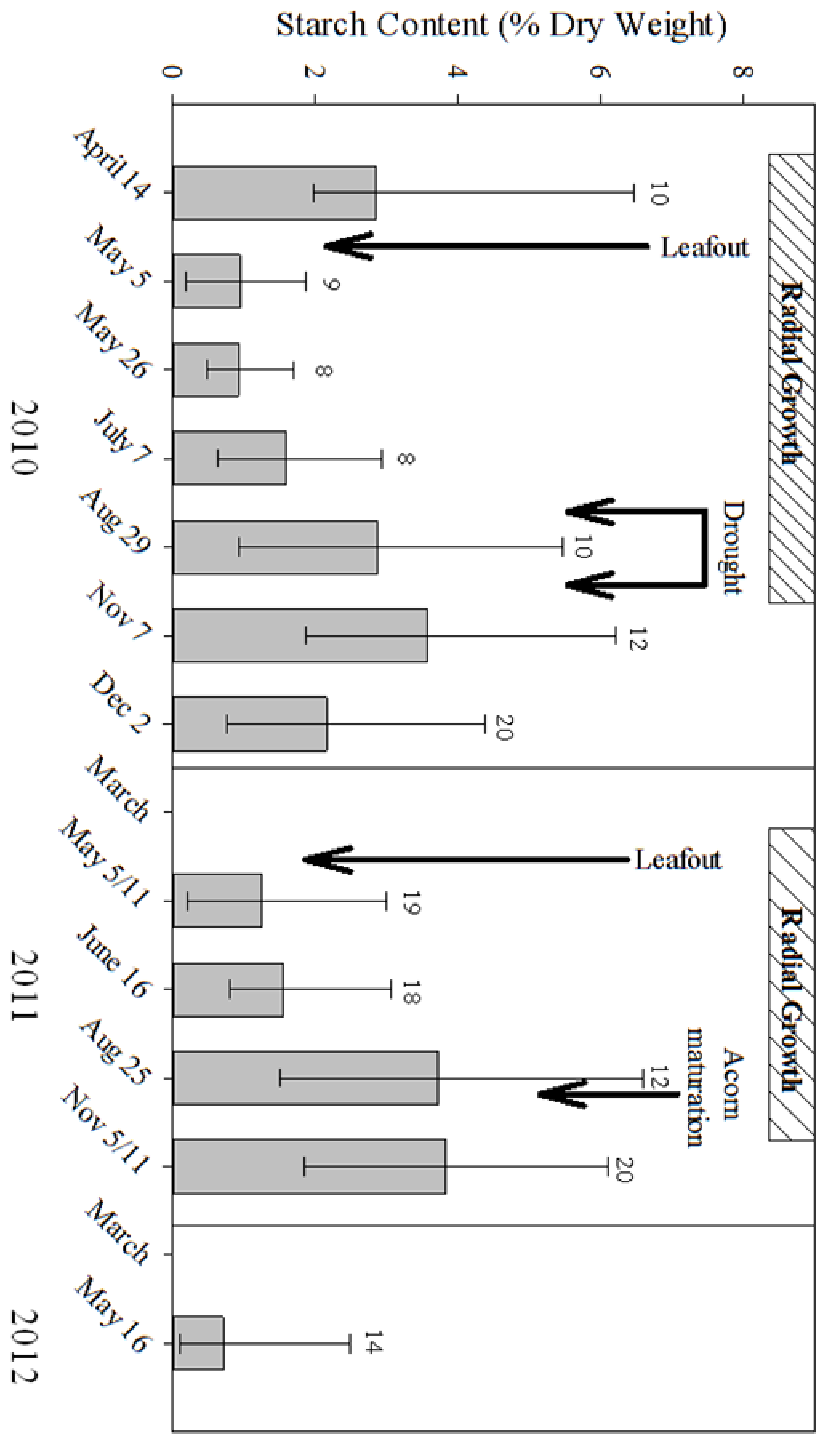

Starch Content (% Dry Weight)
0
2
4
6
8
April 14
May 5
May 26
July 7
Aug 29
Nov 7
Dec 2
March
May 5/11
June 16
Aug 25
Nov 5/11
March
May 16
2010
2011
2012
10
9
8
8
10
12
20
19
18
12
20
14
Leafout
Drought
Leafout
Acorn maturation
Radial Growth
Radial Growth

**Figure 3.5: Seasonal and among-tree variation in stemwood starch levels**. Average starch levels of youngest 2 cm of xylem are shown from April 2010 to May 2012, with error bars representing the maximum and minimum levels. Numbers indicate the sample size for each date. The timing of relevant events is also indicated.

**Table 3.2**: **NSC level and increment comparisons between years**

| | **2010** (% Dry Wt) | **2011** (% Dry Wt) | **N** | **P** (1-tailed) | **P** (2-tailed) |
|---|---|---|---|---|---|
| **May Levels** | | | | | |
| *Starch* | 0.9 | 1.2 | 14 | 0.28 | 0.55 |
| *SS* | 0.7 | 0.7 | 14 | 0.50 | 0.99 |
| *NSC* | 1.6 | 1.8 | 14 | 0.33 | 0.67 |
| **August Levels** | | | | | |
| *Starch* | 2.9 | 3.6 | 6 | *0.052* | *0.10* |
| *SS* | 1.7 | 1.3 | 6 | **0.010** | **0.020** |
| *NSC* | 4.6 | 5.2 | 6 | 0.20 | 0.40 |
| **November Levels** | | | | | |
| *Starch* | 3.3 | 4.1 | 11 | *0.087* | 0.17 |
| *SS* | 1.6 | 1.4 | 11 | **0.022** | **0.043** |
| *NSC* | 5.1 | 5.4 | 11 | 0.24 | 0.49 |
| **May-Aug Increment** | | | | | |
| *Starch* | 1.8 | 2.3 | 5 | 0.29 | 0.58 |
| *NSC* | 2.8 | 3.2 | 5 | 0.42 | 0.83 |
| **Aug-Nov Increment** | | | | | |
| *Starch* | 0.8 | 0.7 | 3 | 0.47 | 0.93 |
| *NSC* | 0.8 | 0.2 | 3 | 0.48 | 0.96 |
| **May-Nov Increment** | | | | | |
| *Starch* | 2.2 | 3.0 | 9 | *0.098* | 0.20 |
| *NSC* | 3.2 | 3.6 | 9 | 0.24 | 0.48 |

Values are the average NSC content of the outermost 2 cm of xylem of all trees sampled. Paired t-tests were performed on the subset of trees that were measured at a given date in both years. N and p-values for both one- and two-tailed t-tests are reported.

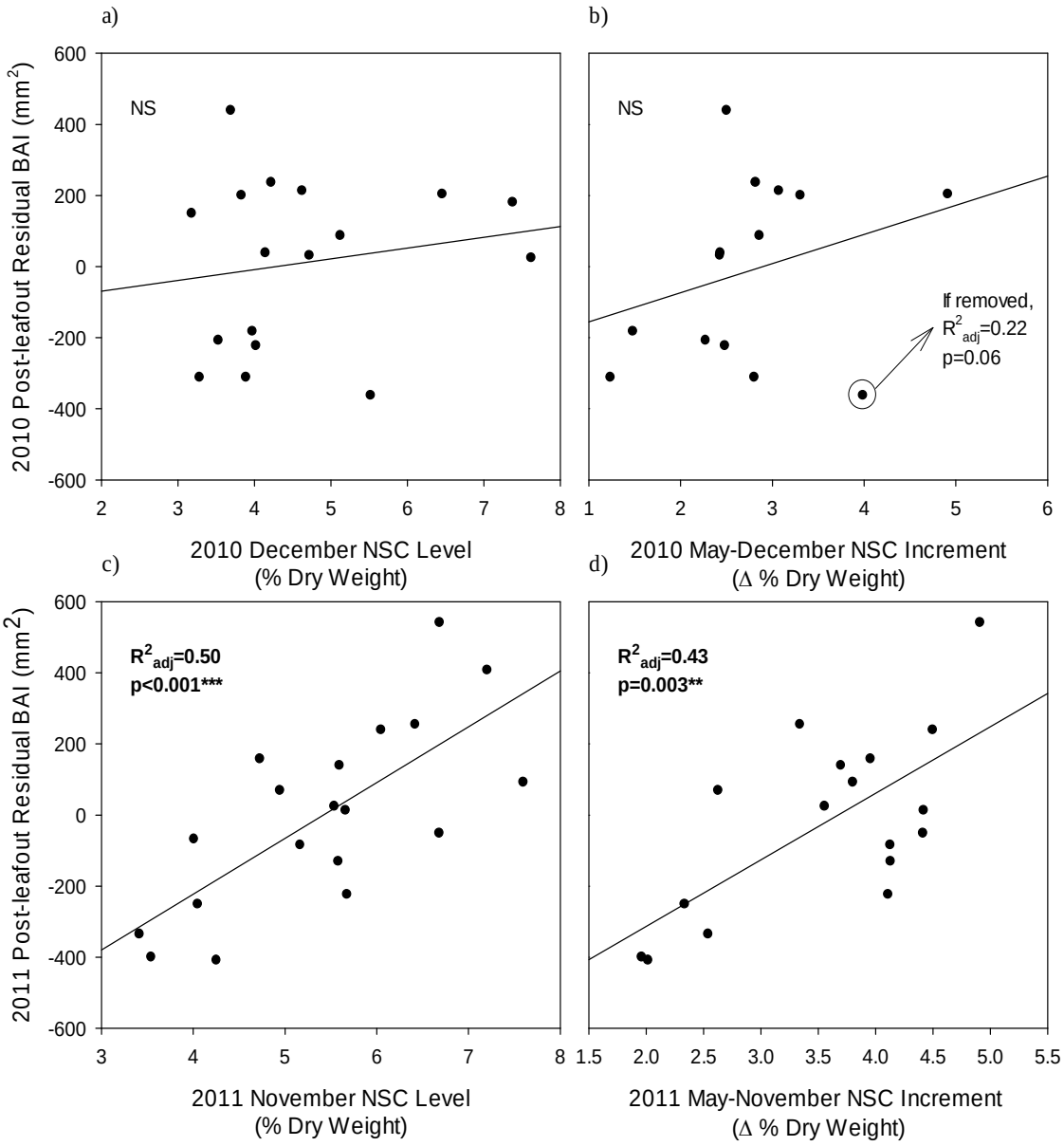



**Figure 3.6: Post-leafout growth vs NSC levels or increments**. a-b) 2010 post-leafout residual BAI vs 2010 December NSC level and vs 2010 May-December NSC increment. c-d) 2011 post-leafout residual BAI vs 2011 November NSC and vs May-November NSC increment.

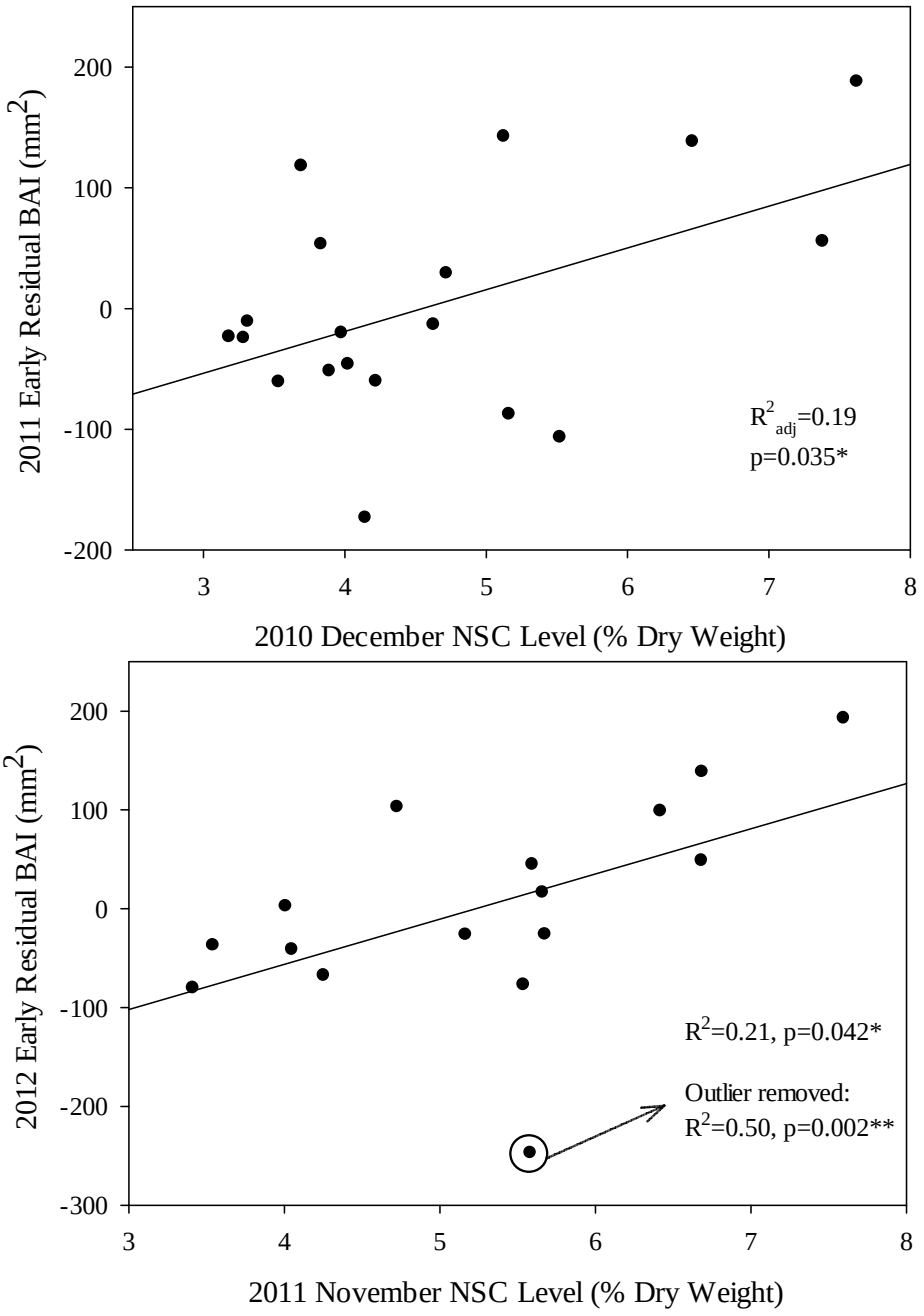



**Figure 3.7**: **Early growth vs. previous year's fall NSC levels**. a) 2011 pre-leafout residual BAI vs 2010 December NSC level and b) 2012 pre-leafout residual BAI vs 2011 November NSC level.

**Table 3.3**: **Regression analyses for May NSC levels vs. early residual BAI, the previous year's late fall NSC levels, and preceding winter decline (previous fall's NSC- current May NSC level)**

| May NSC | vs. Early Residual BAI | | vs. Last Year Fall NSC | | vs. Winter NSC Decline | |
|---|---|---|---|---|---|---|
| | $R^2_{adj}$ | p | $R^2_{adj}$ | p | $R^2_{adj}$ | p |
| **2010 NSC** | (-) 0.13 | 0.11 | ---- | ---- | ---- | ---- |
| **2011 NSC** | **(+)** 0.17 | *0.053* | **(+)** 0.53 | **<0.001** | 0.0 | 0.53 |
| T32 removed | 0.0 | 0.43 | **(+)** 0.25 | **0.029** | ---- | ---- |
| **2012 Starch** | **(+)** 0.28 | **0.029** | **(+)** 0.28 | **0.037** | 0.0 | 0.97 |
| T32 removed | 0.0 | 0.34 | 0.0 | 0.47 | ---- | ---- |
| T32 ,9 removed | ---- | ---- | **(+)** 0.32 | **0.041** | ---- | ---- |

# Chapter 4: Understanding the Role of Allocation Shifts in Carbon Limitation: The Effects of Defoliation on Growth, Storage, and Reproduction


## Abstract

Carbon allocation to growth, storage, and reproduction and the effects of stress and disturbances on these processes remain poorly understood in trees. Nonstructural carbohydrates (NSC) are an important component of the carbon storage pool, and are commonly used to assess whether tree growth under various conditions is carbon limited. Carbon limitation is generally assumed to be the result of decreased carbon uptake, resulting in reduced NSC levels and allocation; however changes in allocation priorities that favor NSC storage could also cause growth to become carbon limited. We defoliated six mature oak trees in 2010 in the New Jersey Pine Barrens and explored how defoliation affects the relationship between radial growth and NSC storage. We also monitored flower and acorn production in 2011, as changes in reproduction allocation could also affect allocation to growth and storage.

Defoliation reduced radial growth for three years. Aboveground starch levels were initially reduced, but starch allocation was never significantly reduced in any year. In addition, belowground NSC allocation was significantly greater in defoliated trees, which allowed belowground stores to recover to control levels by the end of 2011. Defoliation had mixed effects on reproduction in 2011: 2$^{nd}$ yr acorn production was not significantly affected, but no defoliated tree produced any flowers, in stark contrast to undefoliated trees. Defoliation also changed the relationship between NSC increment

and growth in the two years following defoliation, causing a shift in allocation that favored storage more relative to growth. However, the positive relationship between growth and NSC increment indicated that the allocation shift was not due to sink limitation. We conclude that storage is a high priority sink for carbon in these trees, and that carbon limiting events like defoliation can greatly alter carbon allocation in a way that increases the priority of storage. It should be recognized that carbon limitation to growth can occur by two different means: 1) a decrease in plant carbon availability and 2) allocation shifts that may reduce the priority of growth relative to other processes such as storage.

## Introduction

Forest ecosystems have the potential to both mitigate and exacerbate climate change as they control large fluxes of carbon, water, and energy into and out of the atmosphere. Forests have already greatly reduced rising $CO_2$ levels from anthropogenic carbon emissions, in part from enhanced tree growth (Myneni et al. 2001, Luyssaert et al. 2008, Pan et al. 2010). It is unclear whether these systems can continue to take up and sequester more carbon, though. A multitude of other global change factors, such as rising temperatures, more frequent insect outbreaks, changing precipitation regimes, and their interactions may greatly impact tree growth and survival in the future, reducing forests' ability to sequester carbon.

One source of uncertainty regarding how tree growth and mortality will respond to different global change scenarios is that we don't know when tree growth and survival

are carbon limited (Korner 2003, Hyv□nen et al. 2007, Sal et al. 2010, McDowell 2011). We often divide mechanisms limiting growth and survival into two categories: carbon and sink limitation. Carbon limitation occurs when growth or survival is limited by the availability of carbon; sink limitation occurs when growth or survival is limited by a tree's ability to use the available carbon for growth due to such factors as insufficient nutrient supply, direct environmental constraints on the rate of biosynthesis (e.g. temperature), or developmental limits. The importance of this dichotomy to global change is that if trees are carbon limited under certain conditions, such as drought, greater carbon uptake in response to elevated $CO_2$ levels should enhance tree growth (or survival) and forest carbon sequestration. If trees are sink limited, enhanced carbon uptake will have no effect (Korner 2003).

Nonstructural carbohydrate (NSC) levels or dynamics are often used to help distinguish between carbon and sink limitation, particularly under stressful conditions. When comparing growth under stressful and less stressful conditions, a sink limitation to growth is often inferred if NSC concentrations are not lower in the stressful condition. The rationale for this inference is that if growth was carbon limited, NSC stores would be used to supplement photosynthesis, but if NSC levels do not decline under stress, growth must be carbon saturated and limited by some other factor. Because many stresses that reduce growth also increase or do not affect NSC levels (e.g. Korner 2003, Palacio et al. 2008, Sala & Hoch 2009, Sanz-Perez et al. 2009), many have concluded that tree growth under most conditions is sink limited (Korner 2003, Millard et al. 2007).

However, it is possible that NSC levels may not always decline when growth becomes more carbon limited. Increasing NSC levels may be a strategy for avoiding carbon starvation or hydraulic failure which may be more likely under stressful conditions (Smith & Stitt 2007, McDowell & Sevanto 2011, Sala et al. 2012, Wiley & Helliker 2012). If increases in active storage—storage that occurs at the expense of potential growth—are possible, then growth may become more carbon limited in two ways. First, carbon uptake may decrease, and therefore decrease carbon available for growth; this is the cause of carbon limitation that is most usually considered. Second, allocation priorities may change, and growth may become a lower priority sink (Wiley and Helliker 2012). This second cause of carbon limitation is less often acknowledged, but explains how NSC allocation and levels may increase when growth is carbon limited.

Defoliation from insect outbreaks, which can greatly reduce ecosystem productivity (Clark et al. 2010) and cause substantial tree death (Davidson et al. 1996, Hartmann and Messier 2008, Schafer 2011, Palacio et al. 2012), may become more frequent as the climate changes (Ayer & Lombardero 2000, Logan et al. 2003). Understanding how defoliation limits growth will be necessary for predicting how trees will respond to both changes in defoliation intensity, duration, and frequency, as well as how defoliation will interact with changing climatic conditions. Following severe defoliation (or browsing), growth and NSC levels initially decline (Ericsson et al. 1980, Langstrom et al. 1990, Vanderklein & Reich 1999, Li et al. 2002), but NSC levels are often found to recover, or even increase above control levels before growth recovers (Reichenbacker et al. 1996, Kosola et al. 2001, Palacio et al. 2008, Susiluoto et al. 2010,

Palacio et al. 2012). This initial reduction in NSC levels is thought to indicate carbon limited growth, but the recovery of NSC levels is thought to demonstrate that growth becomes sink limited soon after defoliation. However, this interpretation ignores the possibility that growth may be carbon limited by allocation shifts that reduce the priority of growth as a carbon sink (Wiley & Helliker 2012, Chapter 2).

By looking at how defoliation affects the relationship between growth and NSC allocation, we can better determine how a given stress, such as defoliation, limits growth. Under relatively unstressed conditions, growth and NSC allocation may increase together as carbon availability increases (4.1a). If defoliation imposes a carbon limitation to growth, we expect it to be manifested in one of two ways. First, growth may decline simply due to a reduction in carbon uptake. In this case, defoliated trees have less carbon available for both growth and storage, and so both decline. While defoliated and undefoliated trees differ in both growth and storage, the relationship between growth and NSC allocation is not different (Figure 4.1b). Second, defoliation may decrease growth because of an allocation shift by which storage becomes a greater priority relative to growth. In this case, we expect defoliation to change the relationship between growth and NSC allocation so that trees grow less for a given amount of NSC allocation (Figure 4.1c). As a result of this allocation shift, defoliated trees will grow less but store more, but growth will still increase as carbon availability increases. A third possibility for carbon limitation, is that both reduced carbon uptake and allocation shifts occur, leading to reduced growth and reduced or static NSC allocation (Figure 4.1d).

If defoliation imposes a sink limitation, we also expect to see an allocation shift, manifested in one of two ways. First, defoliation may cause growth and NSC allocation to become uncorrelated because as carbon availability increases, defoliated trees cannot grow more, they can only passively store more (Figure 4.1e). Alternatively, defoliation may cause a negative correlation between growth and NSC allocation (Figure 4.1f). In this case, trees that are more sink limited can only store more. In contrast, trees that are less sink limited can grow more, and so less carbon passively accumulates as NSC.

In this study, we hand-defoliated six mature black oaks and compared their growth and NSC responses to those in undefoliated trees over a three-year period. While defoliation experiments are relatively common, our study is apparently the first to explore how defoliation affects the relationship between radial growth and NSC storage. By doing so, we will better able to determine 1) whether defoliation imposes a sink or carbon limitation and 2) if defoliation induces carbon limitation, whether it is caused by reduced carbon uptake, allocation shifts, or both.

Finally, we also explored the effects of defoliation on reproduction and how reproduction, in turn, might have affected the relationship between growth and storage. Reproduction can be a substantial carbon sink in mature trees that may negatively affect carbon allocation to other processes (Genet et al. 2010).

## Materials and methods

*Site Description*

The study was conducted at the Rutgers University Pinelands Research Station in the Pine Barrens of Burlington County, New Jersey. The soil, characterized as a podzol, is sandy with low nutrient concentrations and pH (Tedrow, 1986, Ehrenfeld et al., 1985, Schafer et al. 2011). The climate is cool temperate, ranging from a monthly average of 0.3º C in January to 24.3º C in July (Clark et al. 2012). Annual precipitation is 1123 mm. Precipitation is generally distributed equally throughout the year, though late summer droughts are common. During our study, the 2010 growing season received less than half the rainfall received in 2011 (Song et al. unpublished).

The site is characterized as upland oak/pine forest, with a mixture of chestnut oak (*Quercus prinus*), black oak (*Q. velutina* Lam.), scarlet oak (*Q. coccinia*), white oak (*Q.alba*), pitch pine (*Pinus rigida*), and shortleaf pine (*P. echinata*) dominating the upper canopy and *Gaylussacia* spp and *Vaccinium* spp. dominating the understory (Skowronski et al., 2007). The forest stand was approximately 96 years old in 2010 with a maximum height of 18.8 m and average height of 8 m (Clark et al. 2010, Schafer 2011). Overstory LAI is approximately 3.6 $m^2/m^2$ (for 2006 in Schafer et al. 2010). Our study focused on black oak adult trees.

*Experimental Defoliation*

In June of 2010, six trees, ranging from 12-29 cm in diameter and 10-18 m in height, located along a dirt road running through the research station's forest, were selected for defoliation. Four of the six trees were as tall as surrounding neighbors, one was shorter but receiving full sun, and the last tree was shorter and in partial shade. All

were located within five meters to the road, which was necessary to ensure complete canopy access with a boomlift. From June 6-19, we completely defoliated (100%) these trees using electric grass shears, excising leaves mid-petiole. All leaves were collected and weighed wet. A subset was taken back to the lab to weigh dry for wet/dry calibration, and the remainder were kept in garbage bags until the fall and then spread out underneath the tree.

At the time of defoliation, seven trees of comparable size, canopy position/sun exposure, density of neighbors, and proximity to the road were identified to be used as controls for all measurements (referred to as 'paired controls'). In addition, up to 20 trees from an additional study (Chapter 3) located at the Rutgers Station were included to increase control tree sample size for radial growth and aboveground NSC measurements only. These additional trees are referred to as 'unpaired controls'.

*Photosynthesis Measurements*

After canopy refoliation in 2010, we measured leaf level photosynthesis on detached branches because we did not have access to the canopy. For a subset of trees (4 defoliated and 4 undefoliated), a sunlit branch from the lower canopy was collected with a pole trimmer on August 29 and 31. The cut end of each branch was immediately submerged under water and recut under water with a razor blade. Branches were kept in water and transferred back to the lab, where they were allowed to equilibrate overnight. Light-saturated photosynthesis ($A_{sat}$) was then measured for 1-3 leaves per branch with the LICOR 6400 and the 6400-02B LED Light Source (LICOR Biosciences, Lincoln,

NE, USA). PAR levels and $CO_2$ concentrations were set to 1400 or 1500 μmol (no significant difference: p=0.26) and 400 μmol, respectively. Measurements were recorded every 10 seconds for five minutes after $A_{sat}$ had plateaued, or every minute for 15 minutes in a few cases when acclimation was very slow, to ensure that $A_{sat}$ had plateaued. Measurements were then averaged for each leaf.

To check that $A_{sat}$ measurements of detached branches displayed similar patterns between treatments as in situ measurements, we measured $A_{sat}$ in saplings from the field site that we had also defoliated or left undefoliated on July 8, 2010. We took measurements at three different dates.

In June 2011, we measured photosynthesis in situ by accessing the canopy with a boom lift. Measurements were taken at PAR 1200 μmol and $CO_2$ 400 μmol. For each leaf, $A_{sat}$ was recorded in one of two ways. 1) If $A_{sat}$ plateaued, the last 3-5 minutes were used. 2) If $A_{sat}$ had not plateaued after 20 minutes, the last three minutes were used. $A_{sat}$ of 3-5 leaves of six controls and six defoliated trees was measured between 800-1600 hours, and average $A_{sat}$ was calculated for each leaf. Because $A_{sat}$ tended to decrease with high temperatures, we excluded measurements where leaf temperature was > 30° C, which reduced our sample size at the tree level to five controls and six defoliated trees.

*Leaf traits and canopy phenology*

To monitor effects of defoliation on nitrogen dynamics and leaf quality, we collected leaves for C and N analysis three times throughout the experiment. First, we used a sample of leaves removed during defoliation in 2010 from the both the sun-

exposed upper and shaded lower parts of the canopy (only for defoliated trees). In August 2010, we collected leaves via pole trimmer from a sun-exposed portion of the lower canopies of both controls and defoliated trees. In June 2011, with the use of boom lift, we sampled leaves from the upper canopy of both defoliated and control trees. Measurements were made with an elemental analyzer (Costech Analytical Technologies, CA, USA) coupled to an isotope ratio mass spectrometer (Thermo-Finnigan Delta Plus, Bremen, Germany) at the University of Pennsylvania.

In 2010 and 2011, we also measured leaf size. In 2010, we measured individual leaf area of leaves collected with a pole pruner for photosynthetic measurements. In 2011, leaves for leaf area measurement were collected while measuring photosynthesis in the canopy. Leaf area was measured with a laser area meter (CID model CI-203, Camas, WA, USA).

We also measured leaf starch levels in 2010 and 2011 as a more integrated measure of net carbon uptake, with the idea that starch levels in leaves, which are not important storage organs, will reflect a balance between leaf level carbon supply and whole plant demand. At each sampling date in 2010, we removed a small branch from the lower canopy with a pole pruner, and collected leaf punches using a metal punch from 4-5 different leaves. On August 31, we collected samples within one hour of sunrise (AM) and sunset (PM). On October 28, we collected samples within one hour of sunset and separated the leaf punches from each tree into green, yellow, and red/brown samples. In 2011, we used a boomlift to collect leaf material within 1.5 hours of sunset (June 25) and sunrise (June 26). All samples were wrapped in aluminum foil and kept on dry ice

until returning to the lab, where they were stored at -80 ºC until processed. Leaf samples were ground in liquid nitrogen, and starch content was measured as described below. Starch content is presented as mg glucose equivalents/$cm^2$ leaf area.

In 2010 and 2011, we attempted to identify any effect of defoliation on the timing of canopy senescence, as we had noticed later senescence in defoliated saplings the previous year. To do this, we visually estimated the percent of each tree's canopy that remained green on October 28, 2010 and on November 2, 2011.

*Radial growth measurements*

Spring-loaded dendrometer bands were installed at breast height (~1.4 m) in June 2010. Changes in circumference were measured with a digital caliper. In 2010, measurements were made on October 28 for growth since July 7. Measurements were made on May 5, June 16, July 11, August 25, and November 9 in 2011 and on May 16 and November 15 in 2012.

For each tree, change in circumference ($\Delta C$) over a given period was converted into basal area increment (BAI) by the following equation:

$$BAI = ((C_{init} + \Delta C)/2\pi)^2 - (C_{init}/2\pi)^2$$

where $C_{init}$ is the initial circumference for the beginning of the current year. For 2010, $C_{init}$ was the circumference as measured by DBH tape. For 2011 and 2012, $C_{init}$ was calculated as the sum of the previous year's $C_{init}$ and the previous year's total $\Delta C$.

We compared BAI among trees using Residual BAI as a measure of growth for each time period, as described in Chapter 3. To calculate Residual BAI, we regressed BAI for a given time period against initial basal area (BA) and saved the residuals from this analysis. We used the residual for each tree, or Residual BAI, as a comparable measure of growth; residual BAI measures the amount and direction of deviation from expected growth, given the tree's size. This measure therefore removes variation due to size. For 2011, we also used a similar procedure to compare aboveground volume increment (estimated as the BAI x tree height) between the six defoliated trees and seven controls.

During the experiment, two dendrometer bands were vandalized. The first band was removed between July 11 and August 25, 2011 from a defoliated tree, and the second was removed between the November 11, 2011 and May 1, 2012. In both cases, new bands were installed. However, growth increments for these periods are therefore not known. We did not include these measurements when comparing growth at sub-yearly intervals, but we calculated a replacement value in order to obtain a whole growing season BAI. Replacement values were calculated by first calculating the average ratio of BAI for the missing period to BAI for the remainder of the year for all defoliated trees and then multiplying this average by the BAI for the remainder of the year of the tree with the missing measurement.

*Wood Nonstructural Carbohydrates*

To measure wood NSC levels, we collected tree cores periodically. Because we wanted to relate a tree's NSC levels to its growth, we repeatedly sampled individual trees. Samples were taken between 1.0 and 1.2 m height. Within this region, we did not attempt to standardize the cardinal direction at each date; instead, the location of the core was picked randomly while ensuring that samples were not taken too close together. To minimize exposure of inner tree to pathogens, we sterilized the increment borer with ethanol before taking each sample and plugged the borer hole with wax after core removal. Samples were immediately placed on dry ice until we returned to the lab, where they were stored at -80ºC until processed.

For defoliated trees, a first sample was taken in June 2010 on the day we completed defoliating each tree. The first tree core for all controls was taken on the day we finished defoliating the last tree, June 19. Additional samples were collected on July 7 (mid-refoliation) and November 7 in 2010, on May 5, June 16, August 25, and November 2 in 2011, and on May 16 and November 5 in 2011. The August 25 sample excluded the smallest defoliated and smallest control tree to minimize damage. On May 11 and November 9, 2011, samples were also collected from below the root collar.

From each tree core, we removed the phloem and bark from the outermost 2 cm of xylem (starch levels were found to be near zero at further depths; data not shown). 2-cm segments were divided into 1-cm segments (for greater precision aboveground) or not (belowground), microwaved at 1000 watts for 60 seconds to denature enzymes, and oven-dried at 70ºC for 96 hours.

Following the method of Baud et al (2002), we determined the starch content for all samples and soluble sugar (glucose, fructose, and sucrose) content for a subset of dates. Each sample was ground to powder in a ball mill and extracted twice in 80% ethanol at 80ºC for one hour; supernatants from a sample containing the soluble sugars were removed and pooled. Pellets (containing starch) and supernatants (containing glucose, fructose, and sucrose) were vacuum-dried and then stored at -20ºC. Starch was digested to glucose with α-amylase and amyloglucosidase. Resuspended supernatants were extracted with PVPP to remove phenolic compounds; one aliquot was removed for sucrose determination and the remainder used for glucose/fructose determination. Sucrose was digested by invertase, and fructose was converted to glucose by isomerase. Glucose content was then determined photometrically at 340 nm following digestion with glucose-6-phosphate dehydrogenase and hexokinase, and absorbance was compared to standard curves using pure starch, glucose, fructose, or sucrose. Fructose and sucrose contents were calculated by subtracting actual glucose content. All NSC levels are reported as glucose equivalents percent of dry weight. Soluble sugar levels are the sum of the glucose, fructose, and sucrose percents dry weight.

In addition to starch and NSC levels, we also compared the change in levels from one date to another, denoted as starch or NSC increment; we use this as an estimate of net storage allocation.

*Reproduction*

To determine the effect of defoliation on reproduction, we monitored flower and acorn production in 2011 and 2012. Black oaks flower at leaf emergence in late

April/early May, and acorns mature after two growing seasons. We used binoculars to determine presence/absence of male flowers on each tree on May 5 and 11, 2011 and May 1, 2012. In addition, we noted the presence/absence of acorns. In June 2011, canopy access via boom lift allowed us to determine if trees were producing 1st and 2nd year acorns (1st year "acorns" are unfertilized female flowers; Sork et al. 1993). In July 2012, we determined the presence of 2nd year acorns (pollinated in 2011) using binoculars and a spotting scope. We attempted to quantify 2nd year acorn production in 2011. For five controls and defoliated trees each (the two smallest trees had no 2nd year acorns), we counted the number of acorns along 1-year old branches for between 13-40 branches per tree from the top of each tree's canopy.

*Statistical Analyses*

Data were transformed when residuals were not normally distributed or displayed heteroscedascity. For these variables, data were either ln-transformed (sucrose, glucose, and fructose levels, 2010 $A_{sat}$, leaf size, acorns/stem, post-leafout BAI for 2010/11) or arcsine-square root transformed (stemwood starch levels in ANOVA). In the case of root NSC levels, transformations could not restore residuals to normality. We therefore analyzed the data untransformed using a two-way ANOVA with date and treatment as main effects, and as a precaution, we also ran a permutation test, which does not assume normality. The two analyses provided almost identical results, and we report both.

The effects of defoliation on $A_{sat}$, leaf size, and acorns/stem were determined using ANOVA with a nested design. For August and June leaf starch levels, leaf

nitrogen, and carbon content, treatment effects were determined using univariate repeated measures.

For sugar and aboveground starch levels, treatment differences were analyzed using two-way ANOVAs with date and treatment as main effects. We could not use repeated measures, as individual trees were not all sampled on every date. Instead, we adjusted the denominator degrees of freedom using the Greenhouse-Geisser correction; the estimate of epsilon was obtained by running repeated measures analyses with all defoliated trees and six of the paired control trees which had been sampled on all dates. Differences among treatments for each date were determined using slice tests with degrees of freedom similarly adjusted.

We compared BAI between treatments for growth that occurred until early May, mid-canopy leafout (early BAI) and for the remainder of the growing season (post-leafout BAI) using ANCOVA with initial basal area (BA) as the covariate, after testing for homogeneity of slopes. In one case there was a marginally significant interaction between BA and treatment, and so we fit a single regression line through both treatments and performed an ANOVA on the residuals from this regression. For these analyses, we excluded three unpaired control trees from the other study (see above) that had a larger diameter than that of any paired control or defoliated tree.

Finally, we used ANCOVA to determine the effect of defoliation on the relationship between growth and NSC allocation. Using Residual BAI as the response variable and starch or NSC increment as the covariate, we could determine how growth

and storage were correlated. The direction of this relationship can help to distinguish between carbon and sink limitation, and the test for homogeneity of slopes (i.e. testing the interaction between covariate and treatment) can be used to help determine if defoliated and controls differed in the manner of growth limitation. A significant treatment effect would be indicative of an allocation shift.

Analyses were run in JMP (10.0, SAS Institute, Inc) except for permutation tests which were run in R. All graphs were made in Sigma Plot (12.3,Systat, Inc).

## Results

### *Leaf level measurements*

Defoliation increased leaf-level photosynthesis in 2010, but not 2011. Following canopy refoliation in 2010, $A_{sat}$ was higher for defoliated trees on a leaf area and mass basis, although only significant for the latter ($F_{1,5.87}$=1.50, p=0.27 and $F_{1,6.34}$=6.98, p=0.037; Figure 4.2). Consistent with this trend, in-situ measurements of area-based $A_{sat}$ were also greater, though not significantly, for defoliated saplings in the same year (Appendix Figure A4.1). Leaf starch content in August 2010, while affected by time of day, was not significantly affected by defoliation, although it tended to be lower in defoliated trees at both times (Table 4.1). In 2011, defoliation did not significantly affect $A_{sat}$ on either an area or mass basis (Table 4.2). Leaf starch content in 2011 was again strongly affected by the time of day, but not by defoliation (Table 4.1).

Leaf N content did not significantly differ between control and defoliated trees in 2010 (Table 4.3a), but the lack of difference in 2010 is hard to interpret as leaves of defoliated trees were much younger than control leaves. Leaf age post-defoliation in 2010 was more similar to leaf age at the time of defoliation. In 2010, refoliated leaves tended to have lower N content than leaves removed during defoliation (paired t-test: t=1.92, p=0.057; Table 4.3b). This trend becomes significant when five of six defoliated trees are compared (p=0.018). In 2011, while control and defoliated trees did not differ significantly, there is again a trend of reduced N content in defoliated leaves compared to 2010 pre-defoliation values, although not significant (paired t-test: t=1.53: p=0.093; Table 4.3b).

Defoliation tended to only affect other leaf traits of reflush leaves in 2010, not in 2011. Leaf C content was significantly lower in defoliated trees than in controls in 2010 but not in 2011 (Table 4.3a). $\delta^{13}C$ tended to be more negative (more depleted) in reflush leaves than in controls in 2010, but this difference was only significant when compared with pre-defoliation leaves (Table 4.3a,b). There was no difference in $\delta^{13}C$ in 2011. In 2010, reflush leaves were smaller in area than control leaves ($F_{1,5.8}$=9.98, p=0.021) but leaf size did not differ in 2011 (Table 4.2).

Finally, we detected a difference in the timing of leaf senescence, measured as the percent of canopy that remained green in late October/early November; this difference likely affected late-season carbon uptake. In 2010, defoliated trees retained a significantly greater proportion of green leaves than control trees (p=0.003; Figure 4.3). Leaf starch content at this time was significantly higher in green leaves compared with

senescing leaves, either yellow or red/brown, suggesting greater carbon uptake by defoliated trees at this time (Table 4.1). The trend of later senescence was repeated again in 2011 but was not significant (p=0.20; Figure 4.3).

*Growth*

Following defoliation in 2010, basal area increment (BAI) was reduced relative to undefoliated trees and had not yet recovered to control levels after two full growing seasons at the end of 2012 (Table 4.4). BAI was 60% lower following canopy refoliation in 2010 ($F_{1,26}$=6.36, p=0.018). In 2011, both early season, pre-leafout BAI and post-leafout BAI were reduced by approximately 50% (p<0.001). In 2012, BAI was reduced by 41% and 45% for pre-leafout and post-leafout growth, respectively (p=0.001 and <0.001).

*NSC Storage*

Defoliated trees had reduced stemwood starch levels throughout 2010, the year of defoliation, and starch levels may not have completely recovered by the end of 2012 (Figure 4.4). Starch levels in early July, 2-4 weeks following defoliation, were significantly lower in defoliated trees, and by the end of the growing season, remained about 50% lower than control levels. For the next two growing seasons, starch levels remained lower, though this difference was only significant in August 2011 and marginally significant in November 2012. However, the lack of a significant interaction between date and treatment suggests that defoliated trees may still have been recovering, in terms of starch levels.

While starch levels were negatively impacted by defoliation, starch allocation was not affected as strongly. Starch increment (Δ % starch content) tended to be lower in 2010 and 2012, but these reductions were never significant (Figure 4.5).

In contrast to aboveground starch, greater belowground storage allocation allowed defoliated trees to recover to control NSC levels by the end of the 2011 growing season. Defoliation reduced 2011 root NSC levels in May but not in November, and as a result defoliated trees had a significantly higher belowground NSC increment (Figure 4.7; Date x Treatment effect: p=0.019 for both ANOVA and permutation test). As a result, defoliated trees had a significantly higher belowground NSC increment (Figure 4.6).

Defoliation only had a temporary effect on stemwood total sugar levels but had more lasting impact on the sugar pool components. Total sugar levels (sucrose + glucose + fructose) were significantly lower in defoliated trees in July 2010 due to reduced sucrose levels, following/during canopy refoliation (Figure 4.7). However, by the end of the growing season and in 2011, total sugar levels did not differ between treatments. In contrast to total sugars, there was a significant main effect of defoliation had on individual sugars, as defoliated trees tended to have lower sucrose but higher glucose and fructose levels overall (Figure 4.7). The effect of defoliation was especially strong for glucose and fructose, which remained significantly higher at the end of 2011 when sucrose levels had recovered to control levels.

*Reproduction*

In 2011, most black oaks in our study site were maturing acorns which developed from 2010 flowers. Only one control and one defoliated tree, the smallest in diameter, did not produce any 2nd year acorns. Excluding these trees, there was no significant effect of defoliation on the number of acorns per 1-yr stem, although there was considerable variation in the difference between pairs of defoliated and control trees (Figure 4.8).

Acorn production was not related to growth in defoliated and control trees in the same way. There was a strong positive correlation between residual BAI for 2011 and acorns/stem for controls ($R^2$=0.96, p=0.002) but not for defoliated trees ($R^2$=0). Acorns/stem was positively correlated with May 2011 stemwood starch level when both treatments were combined ($R^2$=0.39, p=0.031). However, acorn production was negatively correlated with May root starch levels across all trees, but with defoliated trees having a lower level of root starch for a given number of acorns/stem (Figure 4.9).

In contrast to acorn production, defoliation had a dramatic effect on flower production in 2011 (Table 4.5). All control trees produced at least some male flowers, while no defoliated tree produced any flowers at all. This stark contrast was apparently repeated for female flowers, as all controls had some 1st year acorns and no defoliated tree had any in 2011; the same pattern again for 2nd year acorns in 2012 confirms this. The effect of defoliation on flowering in 2012 was not as distinct, with 6 out of 7 controls and 3 out of 6 defoliated trees flowering (Table 4.5).

*Growth-storage relationships*

In 2010, defoliation did not affect the relationship between Residual BAI and starch increment, indicating that no significant allocation shift occurred (Figure 4.10a). A single linear regression fit through both treatments demonstrated a positive correlation between Residual BAI ($R^2$=0.28, p=0.026).

In 2011, defoliation caused an allocation shift, significantly affecting the relationship between growth and storage. The significant treatment effect indicates that defoliated trees grew less than controls for a given amount of starch allocation (Figure 4.10b). However, growth and storage were only positively correlated among control trees, not defoliated trees (Figure 4.10b).

Defoliation also caused an allocation shift between growth and belowground storage in 2011, but the relationship between Residual BAI and belowground NSC increment was slightly different from that aboveground. While storage was again favored relative to growth (i.e. treatment effect from ANCOVA: p<0.001), this time growth and storage tended to be positively correlated in both treatments (covariate: p=0.088). This positive correlation became stronger, particularly for defoliated trees, when we used Residual volume increment (BAI * tree height) instead of Residual BAI (Figure 4.11). In addition, for both BAI and volume increment, ANCOVA fits improved when the biggest acorn producer among the controls and the biggest among the defoliated trees were removed. Interestingly, these two trees tended to favor growth, growing more than expected given their root NSC increment.

In 2012, defoliation again led to a shift in allocation that favored storage. While 2012 Residual BAI and starch increment were positively correlated across both treatments (marginally significant), defoliated trees grew significantly less for a given starch increment (Figure 4.10c).

## Discussion

We found that defoliation imposed a carbon limitation to growth, likely as the result of two different effects. First, defoliation reduced whole plant carbon uptake. Second, defoliation caused a shift in the pattern of carbon allocation whereby defoliated trees favored storage allocation at the expense of radial growth. As a result of these changes, growth remained lower for up to three growing seasons following defoliation, which allowed for the complete refilling of belowground stores and complete to partial refilling of aboveground stores.

*Growth limitation the year of defoliation*

Following canopy refoliation in 2010, defoliation intensified the carbon limitation to radial growth by reducing carbon availability (Scenario in Figure 4.1b). The positive correlation between growth and starch increment indicates that tree growth was generally carbon limited, as trees that grew and stored more could only do so if they had more carbon available (Chapter 3). The reduced growth of defoliated trees indicates that growth was even more carbon limited than in controls, and not because of an allocation shift, but likely because of reduced carbon uptake. Reduced growth in response to

reduced carbon uptake is the more classic form of carbon limitation, as NSC levels are also reduced (Korner et al. 2003).

A reduction in carbon uptake in 2010 is supported by several lines of evidence. First, a gypsy moth outbreak at this site only several years before caused LAI to be reduced by 50% following canopy refoliation (Schafer et al. 2010), and so experimental defoliation likely also reduced canopy size, even if not as severely. Second, individual leaves were also significantly smaller after refoliation, a trend that was mirrored by defoliated saplings the same year (data not shown). While defoliated trees did compensate, at least partially, for reduced leaf area through higher leaf-level photosynthetic rates, as commonly seen following defoliation (Hoogesteger & Karlsson 1992, Vanderklein & Reich 1999, Pinkard et al. 2007, Eyles et al. 2011), that leaf starch levels were, if anything, lower in defoliated than in control trees, indicates that at least no overcompensation occurred at the whole canopy level.

Even though total growing season carbon uptake was likely reduced by defoliation, starch increment was not significantly lower in defoliated trees; this may have been accomplished in part by a slight extension of the growing season. Greater leaf starch levels in green leaves, which defoliated trees maintained later into the growing season, are indicative of greater carbon uptake during late fall. Delayed leaf senescence following defoliation has been reported for oaks and birch (Palacio et al. 2013), and we have reported evidence of this phenomenon in black oak saplings (Chapter 2). The cause of the delay is unknown, but may be due to the younger age of refoliated leaves. Alternatively, it may be a mechanism to increase carbon gain when carbon stores are low,

but if so, it comes with an increased risk of losing nitrogen and other nutrients if a frost occurs. Because of this tradeoff, the timing of leaf senescence might be indicative of whether carbon or nitrogen is most limiting.

*Growth limitation in the years following defoliation*

In contrast to 2010, shifts in allocation were largely responsible for the continued growth reductions in 2011 and 2012. In 2012, as in 2010, the positive correlation between growth and storage across treatments indicates that growth was generally carbon limited, but that defoliated trees were more carbon limited. However, in 2012, unlike 2010, carbon limitation was greater in defoliated trees because of an allocation shift (Scenario in Figure 4.1c). This shift was not apparently the result of sink limited growth, as defoliated trees that tended to *store* more also *grew* more, and therefore the defoliated trees that stored most were likely the least carbon limited, but not carbon saturated.

In 2011, while defoliated trees also underwent an allocation shift, favoring storage, the lack of correlation between growth and starch increment suggests that this shift may be due to sink limitation (Scenario in Figure 4.1e). Palacio et al. (2012) found a negative correlation between branch elongation and NSC increment in defoliated pines, which they interpreted as a sink limitation to growth (Scenario in Figure 4.1f). Defoliation may limit growth directly due to nutrient shortage (Tuomi et al. 1990) or indirectly due to hormonal changes that may inhibit growth (Palacio et al. 2012). As a result, when carbon availability increases, the additional carbon can only be shunted

passively to storage. The recovery of NSC levels before growth following defoliation or browsing has often been attributed to this process (Palacio et al. 2008, 2012).

Sink limitations to growth following defoliation may arise due to substantial nitrogen losses (Tuomi et al. 1990), but we did not see evidence of a direct nitrogen limitation on radial growth. While leaf N was significantly lower after defoliation than before, leaf N was never significantly lower than in control levels. Leaf N following defoliation often does not decline or even increases (Langstrom et al. 1990, Palacio et al. 2008, Susiluoto et al. 2010). If N had limited growth directly, we would expect a down-regulation of photosynthesis as a result of N removal from the leaves, but we saw no evidence of this. Leaf N was also not correlated with growth for either treatment.

While the relationship between growth and aboveground storage in 2011 is consistent with sink limitation, the relationship between growth and belowground storage is not. The positive correlation between growth and belowground NSC increment suggest that defoliated tree growth was in fact carbon limited after all, and that growth was reduced in defoliated trees because of greater allocation to belowground storage. It is unclear why this pattern was not also seen aboveground. It may be that for most defoliated trees, aboveground storage requirements were fulfilled, and differences in carbon availability among defoliated trees were only manifested in belowground stores. Following defoliation of aspen, Landhausser and Lieffers (2012) found that belowground NSC levels recovered the year after aboveground levels recovered. They suggested that root storage recovers last because of the greater distance from source organs.

While allocation shifts may largely explain the greater carbon limitation in defoliated trees in the years following defoliation, lower carbon uptake may also have contributed to the growth reduction (the scenario in Figure 1d). Even though, leaf size and $A_{sat}$ were at control levels by 2011, there are several factors indicating that whole plant carbon uptake remained lower than that of controls. First, leaf area recovery after defoliation can often take multiple years (Langstom et al. 1990, Kolb et al. 1992, Karlsson & Weih 2003, Galiano et al. 2011). Second, we noticed that a majority of defoliated trees suffered substantial branch dieback during the winter following defoliation. Third, while starch allocation in defoliated trees was increased *relative* to growth, storage allocation—indicated by starch increment—still tended to be lower in 2012. This tendency for both reduced growth and storage allocation suggests reduced overall carbon availability in defoliated trees.

*Reproduction*

Defoliation appeared to have minimal impact on the reproductive cycle (i.e. flowering initiation through acorn maturation) occurring at the time of defoliation. Although defoliation often reduces fruit production or increases fruit abortion (Rockwood 1973, Stephenson 1980, Hochwender et al. 2003, Hoch 2005, Ichie et al. 2005), here defoliation did not significantly affect the number of acorns/stem maturing in 2011, which developed from flowers pollinated in 2010 before defoliation. This lack of reduction has been seen in other species at the branch level (Obeso 1998, Tamura & Hiura 1998, Thalmann et al. 2003) and whole tree level (Treadwell & Cuda 2007).

The ability of defoliated trees to maintain acorn crops similar to undefoliated trees may be due to a combination of branch autonomy, reliance on reserves, and reduced growth. Fruit and flower production are thought to be branch-autonomous, with mainly locally produced carbon used build flowers and fruits (Watson & Casper 1980, Obeso 1998, Hoch 2005). In 2011, defoliated trees did not have lower $A_{sat}$ or significantly fewer leaves/stem (see Appendix), suggesting that gross carbon uptake at the branch level may not have been greatly reduced. Therefore, if the branch was the main source of carbon supporting acorns, then the carbon available for acorn production may have been little affected. Of course, some defoliated trees suffered substantial branch death during the dormant season, reducing total canopy size. These canopy losses may have imposed greater relative carbon demands on each individual branch. While we did not see the effect of these greater demands on acorn production at the branch level, reproduction may have been maintained at the expense of both local growth, indicated by the marginally reduced branch diameter (appendix), and radial growth. Finally, NSC stores may have been used to maintain acorn production. Though branches may normally rely only on local sources of carbon for fruit development, defoliation can alter this pattern, requiring stored carbon from other organs to help meet reproductive demands (Obeso 1998, Hoch 2005). In our study, root starch levels in early May 2011 were negatively correlated with acorn number, and therefore may be an important source of carbon for reproduction in defoliated and control trees, at least early in the growing season.

While acorns/stem were not significantly reduced in late June 2011, it is still possible that resource allocation to reproduction was lower. We did not measure any

aspect of seed or fruit quality, but defoliation has often been found to decrease seed or fruit weight (Hoch 2005, Percival et al. 2011) even when seed and fruit number do not decline (Thalmann et al. 2003). Also, it is possible that final mature acorn crop was lower in defoliated trees, as higher rates of abortion could have occurred between late June and early September. In black oak, high rates of fruit abortion can occur in late June and early July (Sork et al. 1993). Finally, given the substantial amount of branch mortality that we observed, while branch level production was not significantly lower, the pattern of total acorn production per tree may have been reduced.

Unlike the 2011 acorn crop, the reproductive cycle that began following defoliation was completely altered, consistent with the resource budget model and other resource-models of mast fruiting and flowering. In these models of masting, flowering occurs when plants have built up enough resources, and when flowering and fruiting successfully occurs, resource levels become depleted and flowering does not occur the next year (Crone et al. 2009, reviewed by Miyazaki 2012). In accordance with this, defoliation resulted in a complete lack of male, and probably female, flowers in 2011. The delayed response of reproduction to defoliation likely has to do with when defoliation can first affect bud type determination (Kaitaniemi et al. 1999). In black oak, female floral buds are formed in late summer of the year prior to flowering (Sork et al. 1993). For 2011 flowers, this corresponds to the months just following defoliation, when tree starch levels were significantly reduced. Flower formation has been found to correlate with NSC levels and decline with shading treatments, suggesting carbon is a critical resource involved in the decision to flower (Miyazaki et al. 2009).

Reproduction is often expected to reduce growth and/or storage by using resources that could have been used for these purposes (Newell 1991, Koenig and Knops 1998, Obeso 2002), but this has been called into question by observations that NSC levels may not decline or may even increase in masting years (Korner 2003). We found a positive correlation between 2011 residual BAI and acorns/stem, but this correlation did not exist among defoliated trees. This difference may arise because of reduced carbon availability or increased belowground storage allocation, making nothing but the minimal level of growth and reproductive output possible. In contrast to effects on growth, there was some evidence that reproduction did strain carbon resources, as early May root NSC levels were negatively correlated with acorns/stem across both treatments. However, neither belowground nor aboveground NSC increment appeared to be affected by reproductive output. One reason for this is that trees may only reproduce heavily when resource levels are high, and so may avoid or mitigate tradeoffs with other processes (Sánchez-Humanes et al. 2011). Alternatively, just as with growth and storage, trees may vary substantially in carbon availability, and while a tradeoff between growth, storage, and reproduction may exist for each tree, variation among trees is driven by differences in resource availability (Chapter 3), leading to a positive relationship between all these processes.

*Slow recovery from defoliation*

While NSC levels are often found to decline initially, they often recover or even increase relative to control/undefoliated levels before growth recovers from defoliation or browsing (Reichenbacker et al. 1996, Palacio et al. 2012); our results only partially

confirm these observations. While we did see that average belowground NSC levels recovered to control levels by the end of 2011 (the end of the 2nd growing season), aboveground starch levels may still have not have recovered by the end of 2012. The fact that November 2012 starch levels in defoliated trees were (marginally) significantly lower, and that there was no significant interaction between date and treatment, suggests that aboveground stores were not yet completely refilled, despite a lack of significant difference in November 2011 starch levels.

Extensive canopy dieback may be one reason for the slow recovery of defoliated trees. Canopy dieback, in general, is common following defoliation and drought (Kosola et al. 2011, Breda et al. 2006, Galiano et al. 2011) and is negatively correlated with NSC levels (Breda et al. 2006). Branch death occurred over the winter, and the cause could be a lack of resources for the canopy to fully rebuild or winter embolism. Winter embolism avoidance and repair rely on sufficient sugar concentrations, which are largely supplied by a conversion of starch reserves (Sauter & Cleve 1994, Ameglio et al. 2004, Chapter 3). If branch autonomy continues into the winter, it may determine which branches die back; branches with the lowest starch stores would be the ones fated for death. The reduction of canopy size due to insufficient carbon may slow recovery, and in extreme cases may lead to a downward spiral in carbon gain (Galiano et al. 2011). Such an outcome may be responsible for the slow deterioration of black oaks at our field site that died in 2010 and 2011, several years after defoliation. All these trees had approximately zero starch content before death (data not shown).

*Tradeoff between growth and survival*

At first, it may seem counter-intuitive that trees would respond to an event that causes a carbon limitation by increasing allocation to storage, reducing growth even further. However, this active reduction of growth makes much more sense when we remember that defoliation also imposes a carbon limitation to survival (Bamber and Humphreys 1965, Webb 1981). The correlation of NSC levels or pool size and survival following defoliation (Webb 1981, Canham et al. 1999, Gleason & Ares 2004, Poorter & Kitajima 2007) suggests that increasing storage allocation following a defoliation event may be a mechanism to reduce the chance of carbon starvation by protecting against further defoliations or secondary stresses. Defoliation is often associated with secondary pathogen infections, which eventually kill the tree (Wargo 1996, Davidson et al. 1996), and increased susceptibility to infection has been found to correlate with reduced NSC levels (Wargo 1972, Matson & Waring 1984, Goodsman et al. 2013). Also, as insect outbreaks can often last for more than one year (e.g. Kaitaniemi et al. 1999, Harmann & Messier 2008). Refilling depleted NSC stores immediately could be critical for surviving multiyear outbreaks and preventing pathogen infections. However the refilling of stores will necessarily require the reduction of carbon allocation to other processes, including growth.

Allocation shifts favoring storage could occur in response to many different stresses besides defoliation. Growth declines coupled with increased NSC levels are commonly reported in response to drought (e.g. Galvez et al. 2011), low temperatures (e.g. Bansal & Germino 2008), nitrogen stress (e.g. Birk & Matson 1986), and rubber tapping (Chantuma et al. 2009). Increased NSC levels may ensure enough carbon is

available to deal with increased damage from low temperatures or rubber tapping (Chantuma et al. 2009, Sveinbjornsson 2000), the chance of increased carbon starvation under conditions like drought or nutrient stress where carbon uptake is generally reduced (McDowell 2011, Wiley & Helliker 2012), or to maintain hydraulic integrity when cavitation and dessication risks increase, such as under drought and freezing temperatures (Sala et al. 2012).

*Conclusion*

We found that defoliation greatly affected carbon allocation in trees, increasing the priority of NSC storage above radial growth, and perhaps above reproduction as well. This shift in allocation demonstrates how growth can become carbon limited, not only by reductions in carbon uptake, but by allocation shifts that reduce the priority of growth under stressful conditions. That such shifts are possible makes it unwise to assume increases in NSC levels are the result of sink limited growth. Instead, high NSC levels are compatible with growth that has been carbon limited by increased allocation to storage, which is potentially an important strategy for surviving harsh conditions. Carbon charged forests worldwide may still therefore have the potential for increased tree growth as $CO_2$ levels rise, enhancing carbon sequestration in these ecosystems.

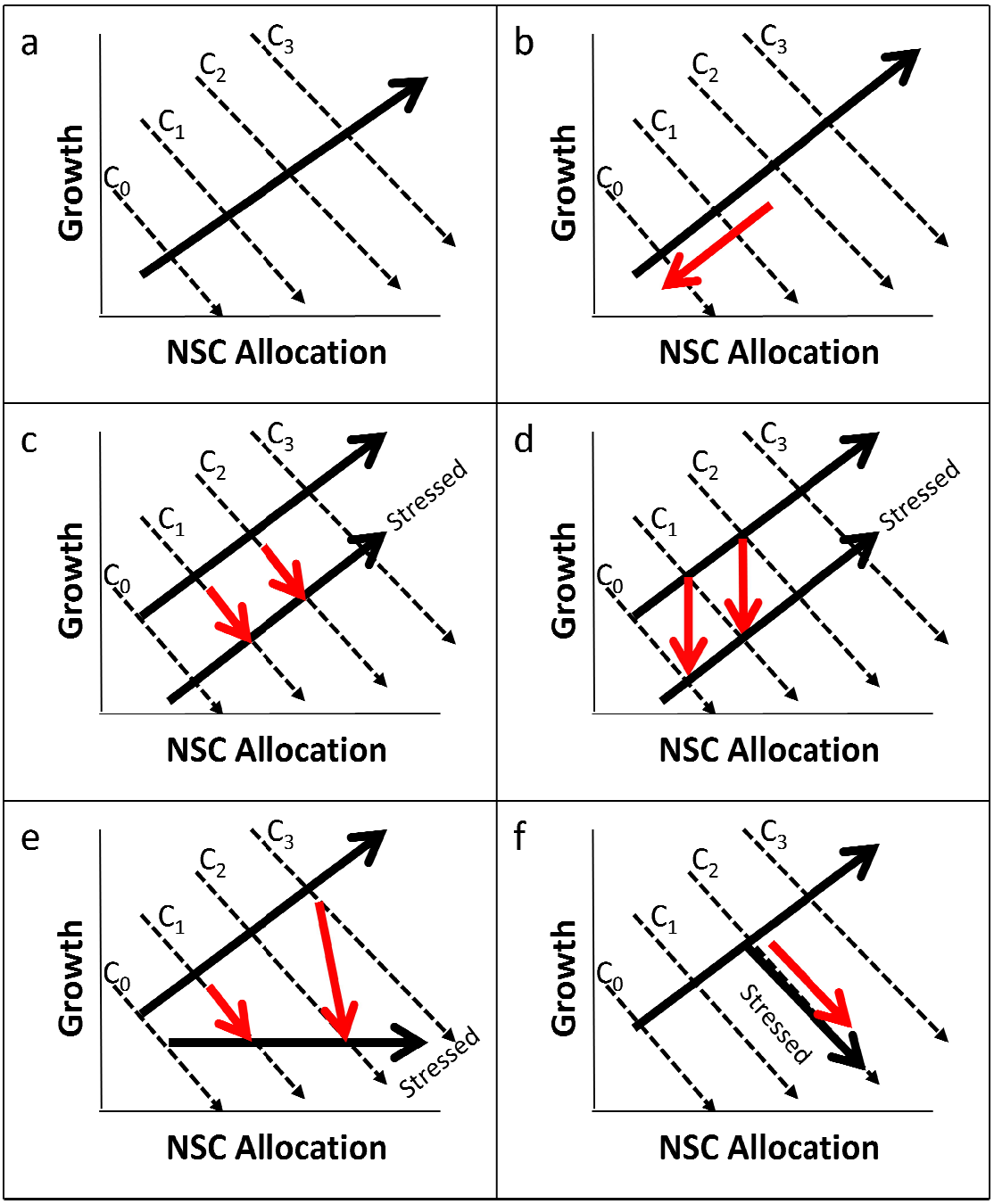



**Figure 4.1. Potential growth and storage relationships in response to defoliation.** Hypothetical relationships are determined by how defoliation limits growth. Black arrows represent growth and storage trajectories as carbon availability increases (i.e.

carbon trajectories). Red arrows indicate the effect of defoliation on carbon availability and on the trajectory itself. **(a)** The unstressed carbon trajectory: as undefoliated trees acquire more carbon, they can shift up to a new carbon isocline ($C_0$ to $C_1$, etc.), where both greater growth and storage are possible. Because growth is not sink limited, there is a positive correlation between growth and NSC allocation. **(b)** Defoliation causes a carbon limitation by reducing carbon uptake, leading to a downward shift along the same trajectory (reduced growth and NSC allocation). **(c)** Defoliation causes a carbon limitation by inducing an allocation shift favoring storage. Defoliated trees shift down the same carbon isocline, onto a new carbon trajectory (reduced growth, increased NSC allocation). **(d)** Defoliation causes a carbon limitation by reducing carbon uptake AND shifting allocation priorities. Trees move onto a lower carbon isocline and a new carbon trajectory (reduced growth, NSC allocation reduced or not changed). **(e)** Defoliation causes a sink limitation. Defoliated trees shift to a new trajectory because growth cannot increase as carbon uptake increases. This leads to no correlation between growth and NSC allocation (growth decreases, NSC allocation increases or is static). **(f)** Defoliation causes a sink limitation, but there is no variation among trees in carbon uptake, only in the degree to which growth is sink limited. This causes a negative correlation between growth and NSC allocation (growth declines, NSC allocation increases).

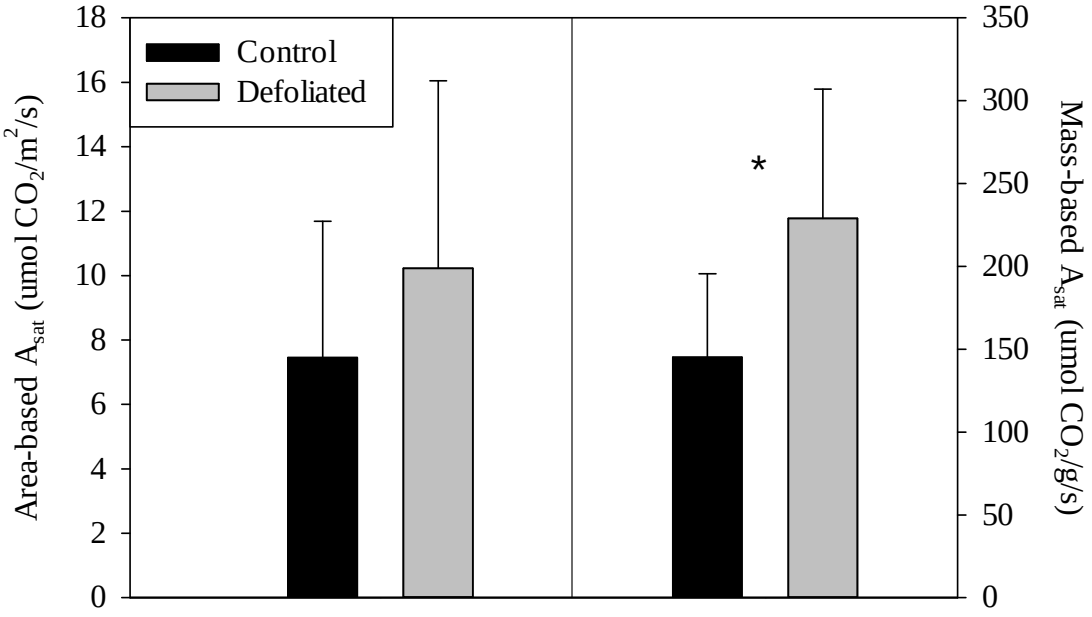



**Figure 4.2**. **2010 leaf-level photosynthetic rates ($A_{sat}$) per area and mass.** Values and error bars are back-transformed averages and 95% confidence intervals, respectively. Mass-based $A_{sat}$ was significantly higher for defoliated trees.

**Table 4.1. Leaf starch levels for 2010 and 2011**

**(a)**

| Leaf Starch ($mg/cm^2$) | **Control** | | **Defoliated** | | p-value | | |
|---|---|---|---|---|---|---|---|
| | *AM* | *PM* | *AM* | *PM* | Treatment | Time | Treat x Time |
| *Aug 2010* | 0.024 | 0.044 | 0.014 | 0.028 | 0.25 | **0.0002** | 0.75 |
| *June 2011* | 0.160 | 0.309 | 0.180 | 0.325 | 0.49 | **<0.0001** | 0.91 |

**(b)**

| Leaf Starch ($mg/cm^2$) | Leaf Color | | | | |
|---|---|---|---|---|---|
| | **Green** | **Yellow** | **Brown/red** | F | p |
| *Oct 2010* | $0.045^a$ | $0.021^b$ | $0.011^c$ | 46.7 | **<0.0001** |

**(a)** Leaf starch comparison between defoliated and control trees. Sample size for June 2011 was 4,4 and 6,6 for Aug 1010; treatment effects were determined using univariate repeated measures. **(b)** Leaf starch comparison between green, yellow, and brown/red leaf area. Leaves were collected from defoliated and control trees on October 28, 2010, the day that % canopy that remained green was estimated. Defoliated trees had significantly higher proportions of green leaves, which had significantly higher starch content than either yellow or brown leaves. Back transformed averages include defoliated and controls trees. Different letters represent significant differences according to Tukey's HSD.

**Table 4.2. 2011 Leaf-level photosynthesis ($A_{sat}$) and leaf size**

| 2011 | Control | Defoliated | F | df | p |
|---|---|---|---|---|---|
| Ave $A_{sat}$/area | 13.9±0.8 | 13.8±0.7 | <0.01 | 1,9.4 | 0.97 |
| Max $A_{sat}$/area | 15.8±0.7 | 15.6±0.6 | 0.07 | 1,9 | 0.80 |
| Ave $A_{sat}$/mass | 201±15 | 202±13 | <0.01 | 1,9.3 | 0.95 |
| Max $A_{sat}$/mass | 227±15 | 230±14 | 0.02 | 1,9 | 0.89 |
| Leaf size ($cm^2$) | 57±4 | 58±4 | 0.02 | 1,9.8 | 0.89 |

Maximum $A_{sat}$ is the average $A_{sat}$ of the leaf with the highest average for each tree.

Averages are given with standard errors.

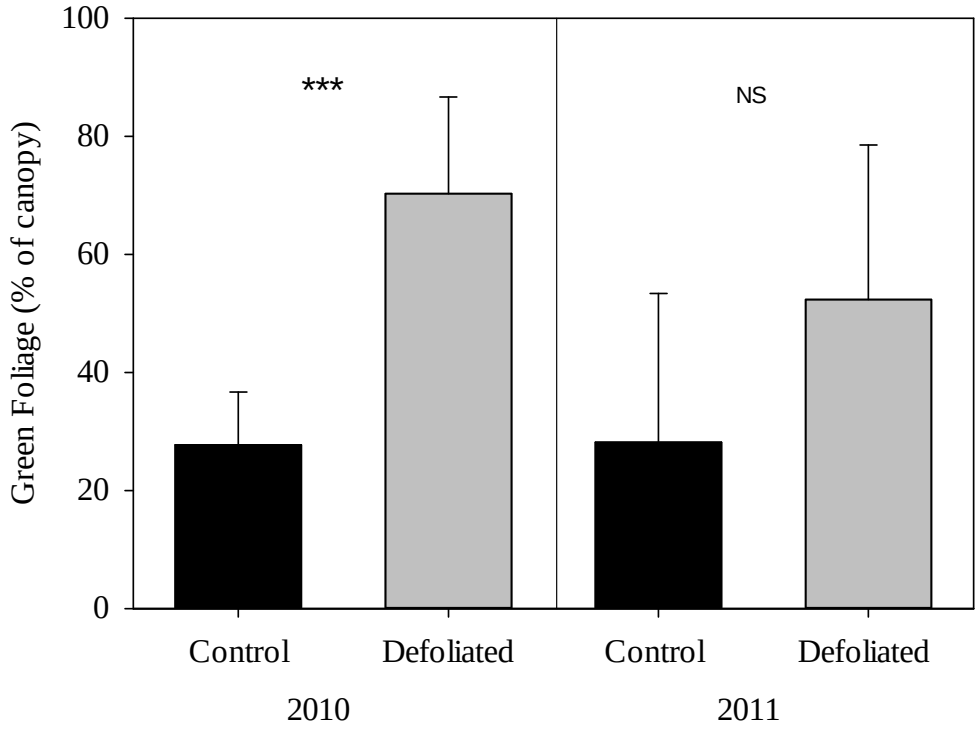



**Figure 4.3. Percent of canopy still green at end of growing season.** For each tree, the percent green foliage was visually estimated on October 28, 2010 (N=27,6) and on November 2, 2011 (N=7,6). Values are back-transformed averages and error bars represent 95% confidence intervals.

**Table 4.3. Leaf carbon and nitrogen for 2010-11**

| **(a)** | **Post-defoliation 2010** | | **2011** | |
|---|---|---|---|---|
| | *Control* | *Defoliated* | *Control* | *Defoliated* |
| **%N** | 1.79 | 1.68 | 1.68 | 1.63 |
| **%C** | **47.2*** | **44.6*** | 47.8 | 47.9 |
| **$\delta^{13}$C** | -29.7 | -30.5 | -28.5 | -28.9 |

| **(b)** | **Pre-defoliation 2010** | | **Post-defoliation 2010** | **Post-defoliation 2011** |
|---|---|---|---|---|
| | *Upper canopy* | *Lower/shaded* | | |
| **% N** | 1.83 | -- | *1.68$^{(*)}$* | *1.63$^{(*)}$* |
| **% C** | 46.1 | -- | 44.6 | **47.9*** |
| **$\delta^{13}$C** | -28.8 | -30.1 | **-30.5**** | -28.9 |

**(a)** Leaf carbon and nitrogen analyses for defoliated and control trees. Averages were compared between controls (N=5) and defoliated trees (N=6) using univariate repeated measures. Within each year, significant differences between treatments were determined using Tukey's HSD and are indicated by asterisks. **(b)** Leaf carbon and nitrogen analyses for defoliated trees. Post-defoliation values from 2010 and 2011 were compared to pre-defoliation values of upper canopy leaves and also of lower canopy leaves for $\delta^{13}$C. * indicate that post-defoliated values were significantly different from pre-defoliated upper canopy values, ** indicate significantly different both upper and lower canopy values, and $^{(*)}$ indicate a marginally significant difference (i.e. $0.05<p<0.10$).

**Table 4.4: Average basal area increment (BAI) adjusted for initial basal area (BA) and ANCOVA results**

| | **Adjusted BAI ($mm^2$)** | | **Treatment** | | **Covariate (BA)** | |
|---|---|---|---|---|---|---|
| | Control | Defoliated | F | p | F | p |
| **2010** | | | | | | |
| *Post-refoliation* | 222 | 87 | 6.36 | **0.018** | 40.8 | **<0.001** |
| **2011** | | | | | | |
| *Early* | 306 | 156 | 22.9 | **<0.001** | 63.4 | **<0.001** |
| *Post-leafout* | 838 | 406 | 33.0 | **<0.001** | 29.4 | **<0.001** |
| **2012** | | | | | | |
| *Early* | 368 | 216 | 13.8** | **0.001**** | NA | NA |
| *Post-leafout* | 770 | 419 | 18.3 | **<0.001** | 13.7 | **0.001** |

**Treatment effect determined by ANOVA of residuals from a single regression of BAI and BA. BAI values are adjusted averages from the ANCOVA with interaction term (i.e. test for homogeneity of slopes).

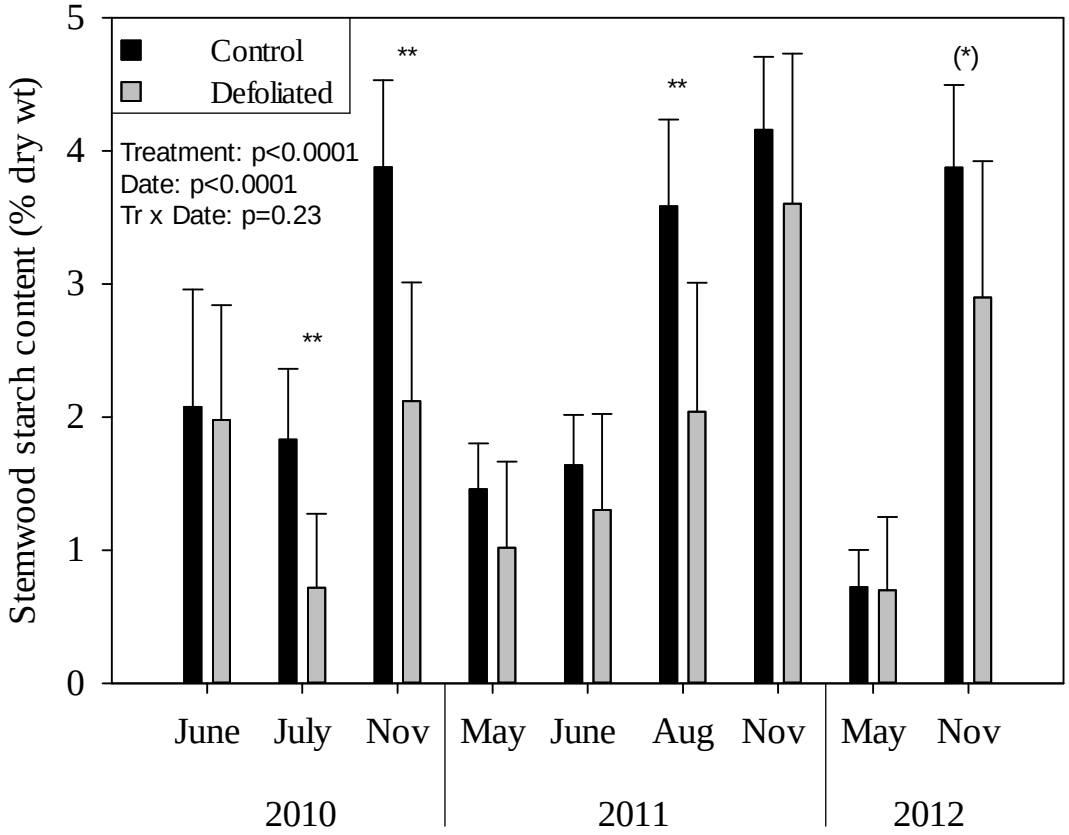



**Figure 4.4**. **Stemwood starch levels for defoliated and control trees**. Starch levels of the youngest 2-cm of xylem were measured at the time of defoliation (June 2010) and over the next three growing seasons. Averages are back-transformed, and error bars represent 95% confidence intervals. Treatment effects were determined by ANOVA after adjusting denominator degrees of freedom. Significant differences are indicated by ** and marginally significant differences are indicated by (*).

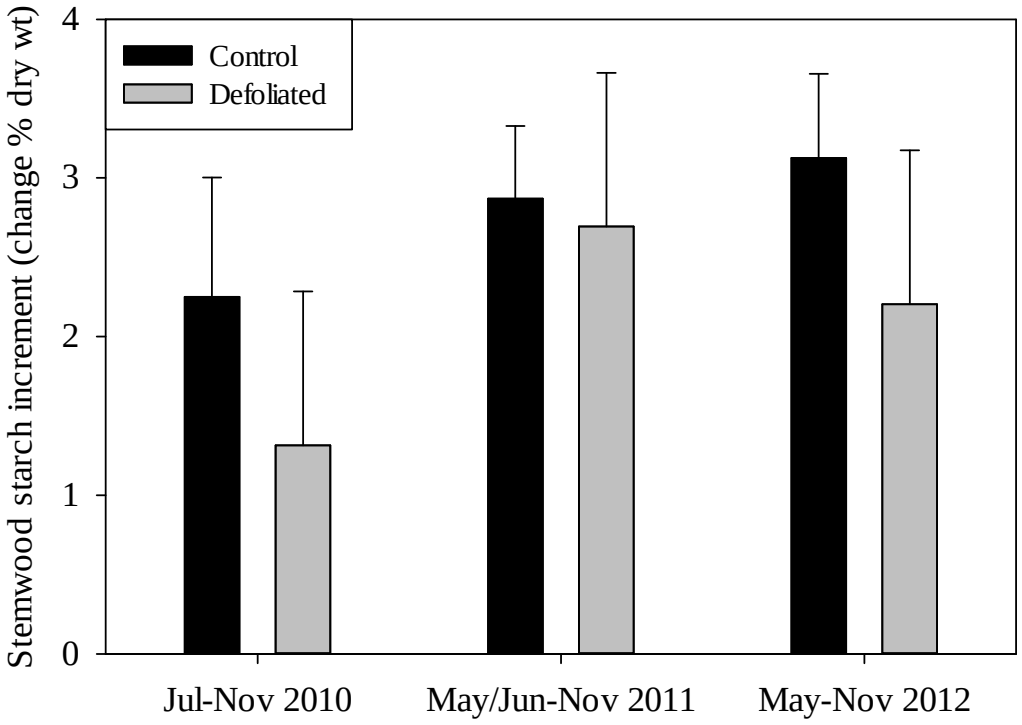



**Figure 4.5. Starch increments for 2010-2011 following defoliation.** Starch increment is an estimate of storage allocation aboveground. For each year, starch increment did not significantly differ between treatments.

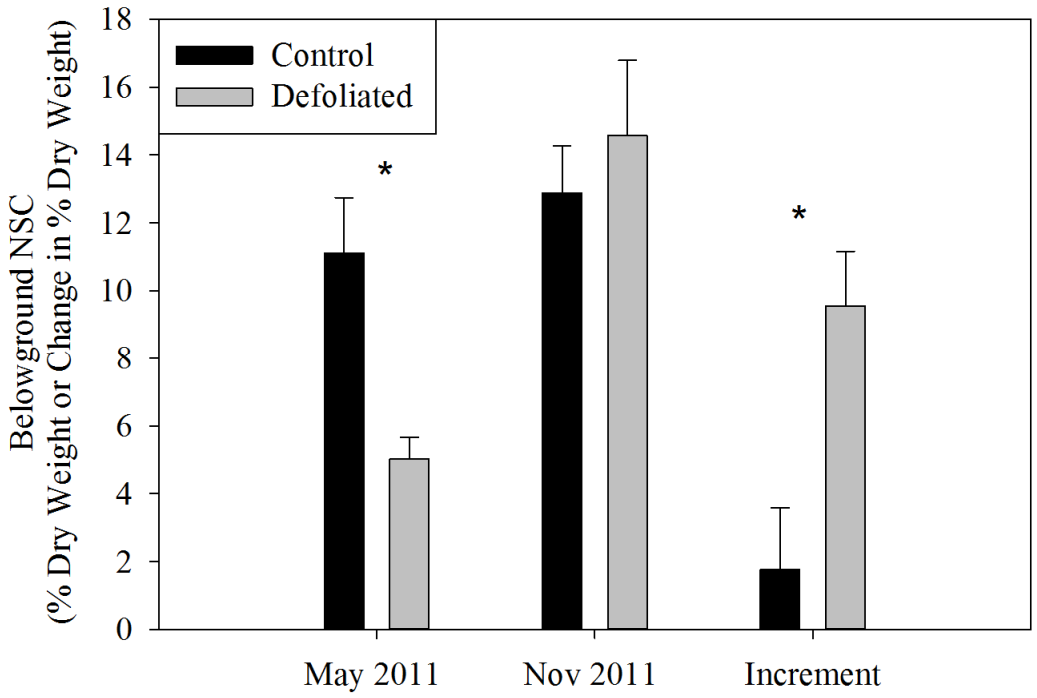



**Figure 4.6. Belowground NSC levels and NSC increment the year following defoliation**. NSC levels are of the youngest 2cm of xylem taken below the root collar in May and November 2011; NSC increment is the change in NSC level between these two dates. Averages and standard errors are for raw data. * indicates a significant effect of defoliation; the significant effect of defoliation on NSC increment is indicated by the significant interaction between treatment and date.

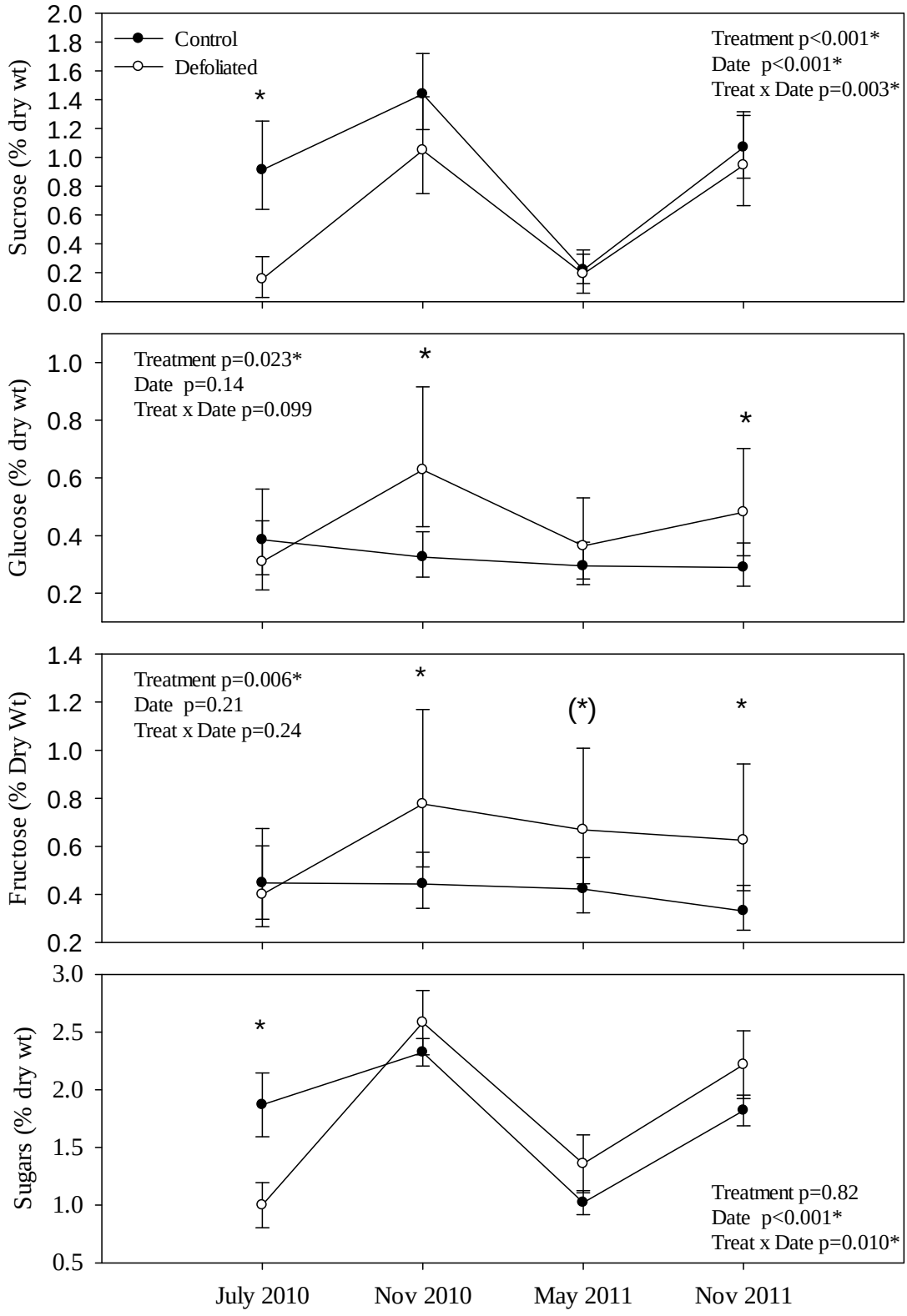

Control
Defoliated
Sucrose (% dry wt)
Treatment p<0.001*
Date p<0.001*
Treat x Date p=0.003*
Glucose (% dry wt)
Treatment p=0.023*
Date p=0.14
Treat x Date p=0.099
Fructose (% Dry Wt)
Treatment p=0.006*
Date p=0.21
Treat x Date p=0.24
(*)
Sugars (% dry wt)
Treatment p=0.82
Date p<0.001*
Treat x Date p=0.010*
July 2010
Nov 2010
May 2011
Nov 2011

**Figure 4.7. Stemwood sucrose, glucose, fructose, and total sugar content**.

Treatment average sugar levels of youngest 1-cm of xylem are given for four dates following defoliation. Error bars represent 95% confidence intervals for individual sugars and standard errors for total sugar. Significant differences are indicated by *, and marginally significant differences are indicated by (*).

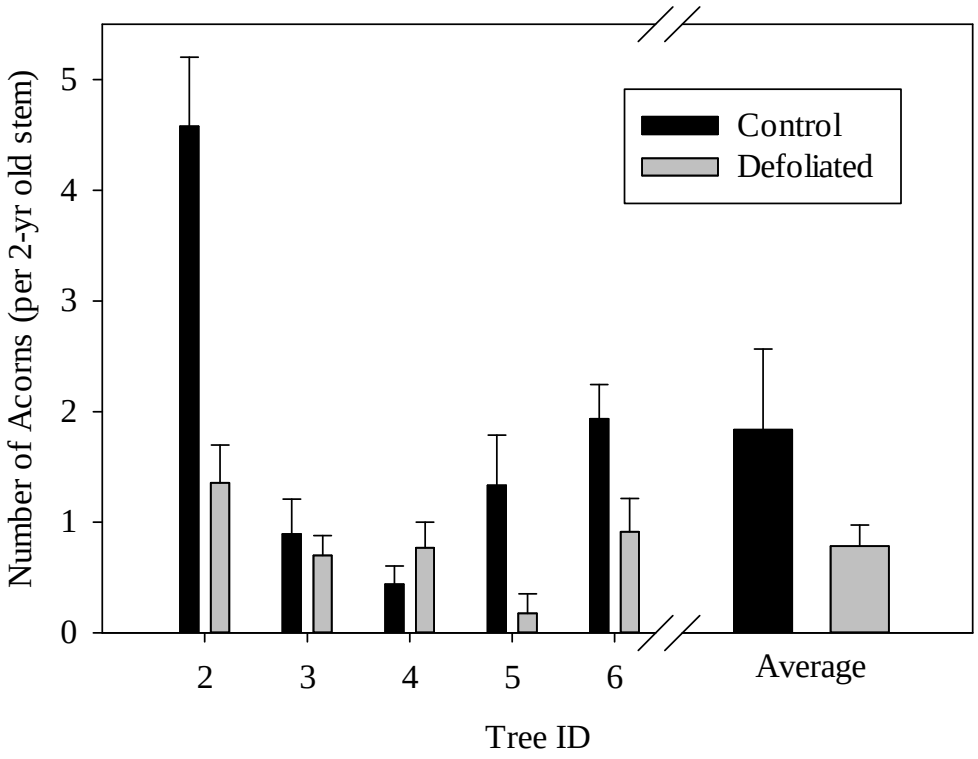



**Figure 4.8. Average number of 2nd-year acorns per 1-yr old branch in June 2011.** These acorns were initiated in 2010 prior to the defoliation treatment. There was no significant effect of defoliation on 2010 acorn crop (p=0.23). Averages and standard errors (error bars) were calculated from the raw data.

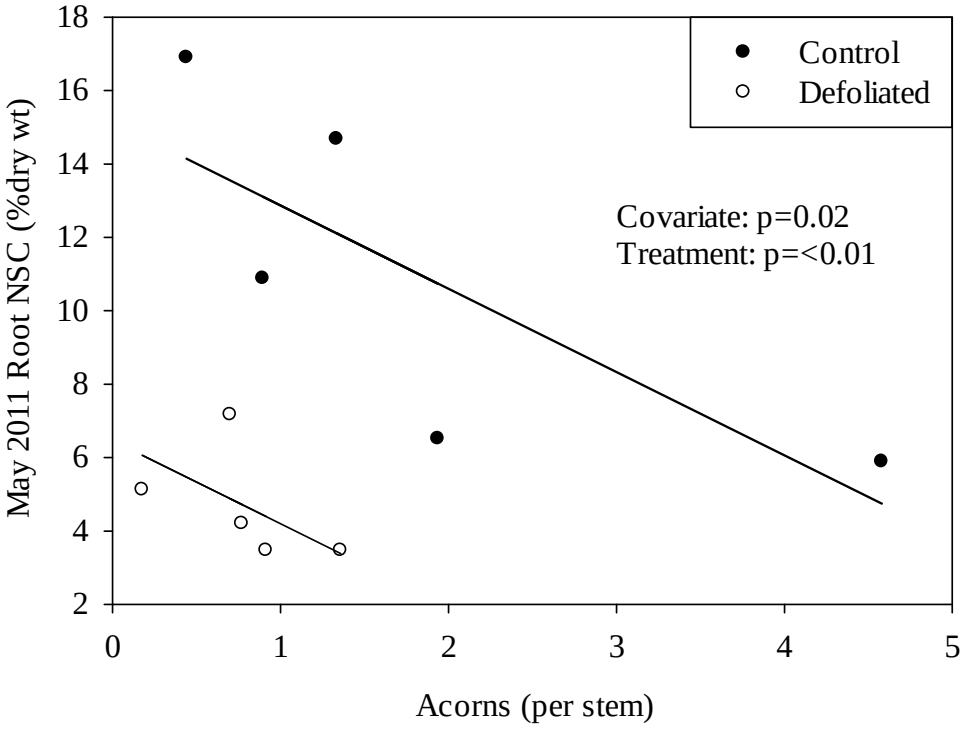


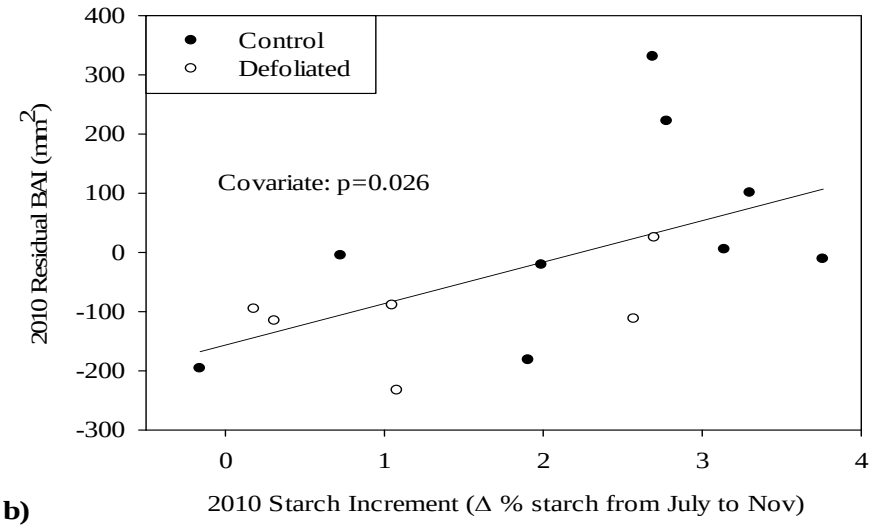

**Figure 4.9. 2011 May root NSC levels as a function of acorn production**. An ANCOVA was used to test if acorn production differentially affected early root NSC levels. Increasing acorn production is associated with lower NSC levels early in the growing season, and for a given acorn production level, defoliated trees have lower NSC. There appears to be a cost in terms of root storage maintenance associated with acorn production, but this cost is greater for defoliated trees. This greater cost is likely related to lower storage levels at the end of the 2010 growing season.

**Table 4.5**. **Presence/absence of flowers and acorns among trees**

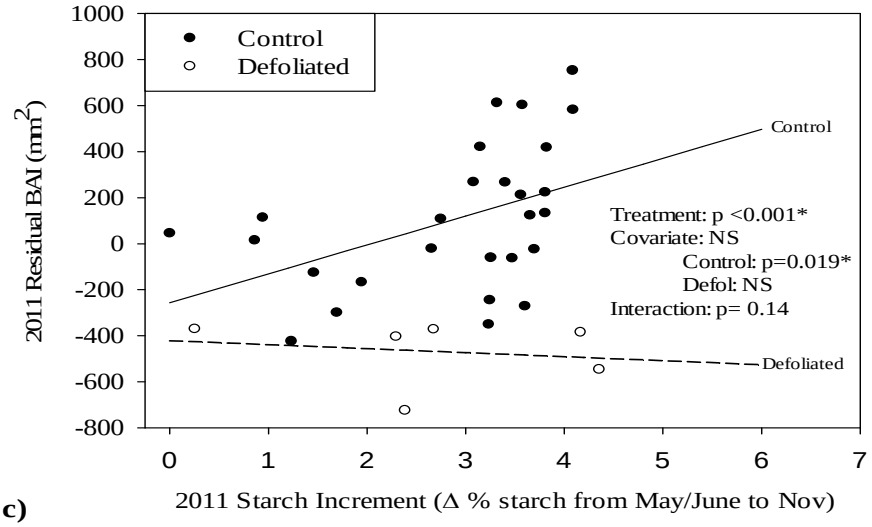
| Presence/ Absence | **Control** | | **Defoliated** | |
|---|---|---|---|---|
| | *Yes* | *No* | *Yes* | No |
| *2011* | | | | |
| **Flowers** | **7** | **0** | **0** | **6** |
| **1st year Acorns** | **6** | **0** | **0** | **6** |
| 2nd year Acorns | 5 | 1 | 5 | 1 |
| *2012* | | | | |
| Flowers | 6 | 1 | 3 | 3 |
| **2nd year Acorns** | **7** | **0** | **0** | **6** |

Trees were considered to be producing flowers or acorns (yes) if at least one flower or acorn was visible. The difference in presence/absence of flowers was tested via contingency analysis using Fisher's exact test. The difference in flower production was significantly different in 2011, and the bolded rows indicate the same flower cohort as they matured.

**a)**


Control
Defoliated
Covariate: p=0.026
2010 Residual BAI (mm2)
2010 Starch Increment (Δ % starch from July to Nov)

**b)**


Control
Defoliated
Control
Treatment: p <0.001*
Covariate: NS
Control: p=0.019*
Defol: NS
Interaction: p= 0.14
Defoliated
2011 Residual BAI (mm2)
2011 Starch Increment (Δ % starch from May/June to Nov)

**c)**

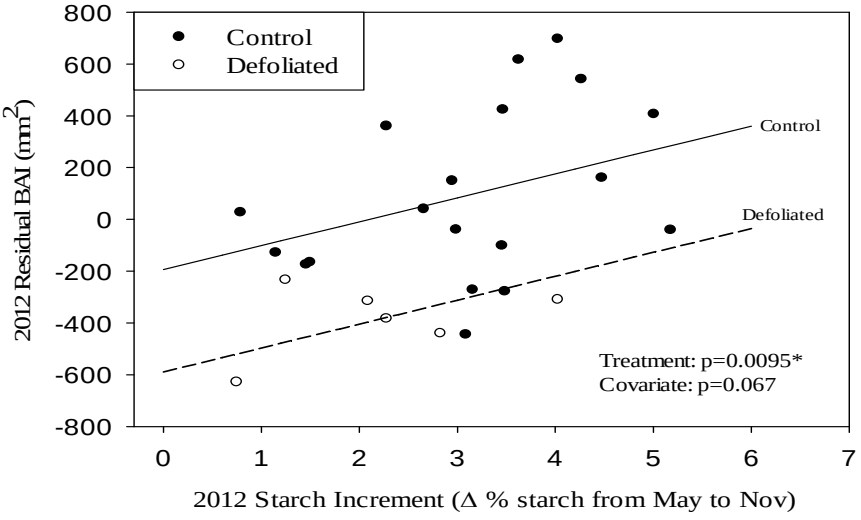

Control
Defoliated
Control
Defoliated
Treatment: p=0.0095*
Covariate: p=0.067
2012 Residual BAI (mm2)
2012 Starch Increment (Δ % starch from May to Nov)

**Figure 4.10. Growth versus storage relationships**. These relationships were explored with ANCOVA of Residual BAI using starch increment as the covariate. **(a)** 2010. Because there was no effect of defoliation in the ANCOVA, a single regression line was fit through both treatments. The lack of defoliation effect indicates that defoliation primarily limited growth by reducing carbon availability, not by shifting allocation. **(b)** 2011. Results from the ANCOVA are shown, but due to the differences in slopes between treatments, regression lines were fit through individual treatments. Allocation is shifted toward storage, but the lack of correlation between growth and storage suggests that growth may be sink limited. **(c)** 2012. Defoliation significantly reduced growth, and it appears to be primarily due a shift in allocation, favoring storage.

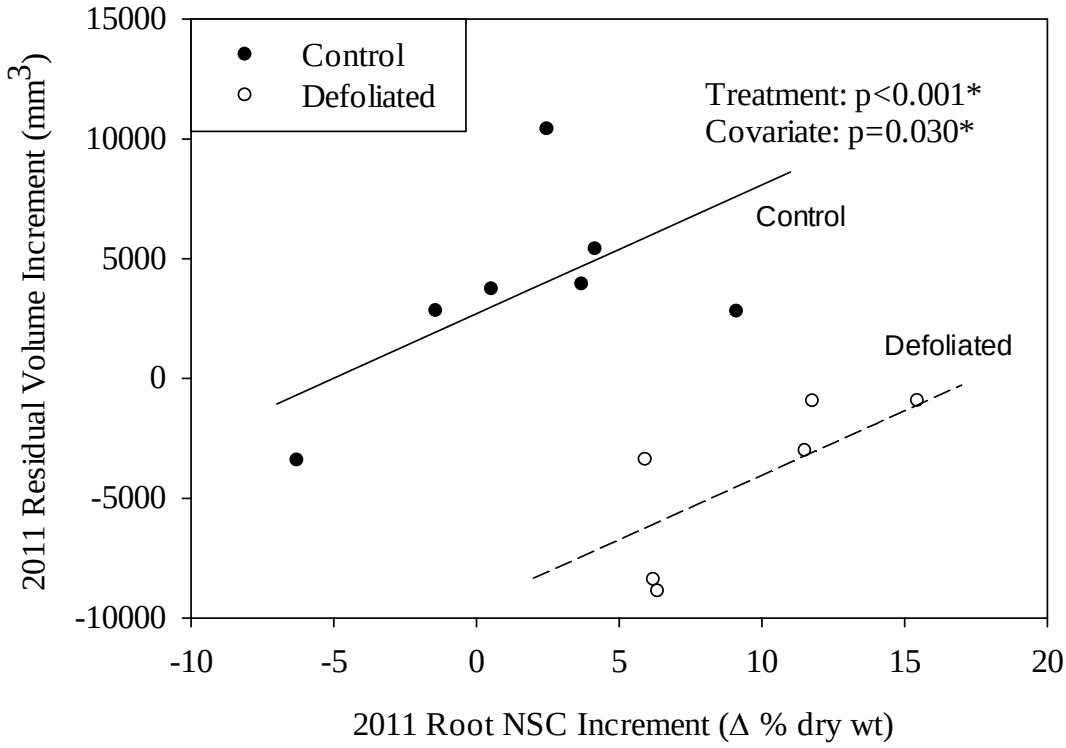



**Figure 4.11. 2011 aboveground residual volume increment versus root NSC increment**. Growth is positively correlated with storage, suggesting that 2011 growth may be carbon limited due to prioritized storage allocation.

# Discussion

Nonstructural carbohydrates (NSC) are an important component of the carbon storage pool in trees, and are commonly used to assess whether tree growth under various conditions is carbon limited. However because NSCs may accumulate passively when growth is carbon saturated or actively at the expense of potential growth, the relationship between carbon limitation and NSC levels may be very complex. Our observations of growth and the NSC dynamics in black oak have resulted in two main findings. First, NSC allocation is largely active, occurring at the expense of potential growth. Second, carbon limitation to growth can result not only from a decrease in overall carbon availability but from shifts in allocation priorities that tend to reduce the importance of growth relative to other functions, such as storage. These findings suggest that storage should be considered a relatively high priority sink in trees, and that its importance relative to growth may even increase when carbon availability declines.

Defoliation has widely been seen as a carbon limiting event (Landhausser & Leiffers 2012), although many have argued that the recovery of NSC levels before growth rates indicates that growth ceases to be carbon limited soon after (Palacio et al. 2008, 2012). In addition, some have assumed that even the initial growth reduction following defoliation is not due to carbon limitation as NSC stores are not completely used up (Ericsson et al. 1980). However, our results suggest that growth following defoliation is sensitive to carbon supply and that the reduction in growth therefore is due to carbon limitation. The fact that NSC levels tend to recover before growth is not a result

of sink limited growth and resulting passive NSC accumulation but instead is due to the increased priority of storage.

An incomplete remobilization of NSC stores following defoliation or at the beginning of the spring for deciduous trees has been seen as evidence that growth is sink limited (Hoch et al. 2003), but, we did not see evidence for this in Chapter 3. Early May NSC levels were not generally or consistently correlated with early growth, but both were instead correlated with late fall NSC levels. These correlations suggest that both early growth and minimum NSC level are determined by carbon supply. As argued in Chapter 1, if NSC stores were completely used up, either following defoliation or spring growth, then trees would be extremely vulnerable if photosynthesis ceased, due to defoliation, fire, or drought. Because the level of NSC stores maintained following remobilization increased with greater carbon availability, trees that are less carbon limited are in a better position to protect themselves against these disturbances.

*Storage allocation in seedlings versus adults*

Storage is often considered a low priority sink in adult trees (Dickson 1989, Le Roux et al. 2001), but it is considered to be a high priority for seedlings of some species (Canham et al. 1999) and an important determinant of seedling ecology. Differences among species in shade tolerance are argued to be the result of a tradeoff between growth and survival (Pacala et al. 1994, Kobe et al. 1995, Seiwa 2007), which may largely be due to a tradeoff between growth and storage allocation (Kobe 1997). Survival in low light or following defoliation is commonly associated with initially higher NSC levels or pool size (Canham et al. 1999, Gleason & Ares 2004, Myers & Kitajima 2007), and NSC levels can be negatively correlated with growth (Poorter & Kitajima, 2007). Species that

are adapted to stressful or disturbance-prone habitats may therefore tend to allocate more heavily to storage.

It may be the case that storage is a higher priority in younger trees than in adults. In adult black oaks, defoliated trees had significantly lower starch levels than undefoliated trees at the end of the first growing season (Chapter 4), but this was not the case for fully defoliated saplings (Chapter 2). The reason for the difference may be that seedlings and saplings favor storage more than adults, allowing storage to recover more quickly. As small trees, saplings may be more prone to total canopy loss as leaves and young branches are within the reach of mammals and small understory fires. Having a much smaller canopy may also make it more likely that a sapling or seedling's whole canopy would be eaten or destroyed. Furthermore, when forest gaps appear, saplings may have to grow faster than current photosynthate supply will allow if they are to reach the canopy before gap closure. As a result, selection for greater storage allocation at the seedling or sapling stage, and not later stages, may have occurred.

However, adult defoliated oaks did appear to favor starch allocation at the expense of growth, and so the slower recovery of NSC levels in adult trees may not have been related to differences in allocation priorities between saplings and adults. First, the slower recovery of adults may arise from the poorer growing conditions in the pine barrens. While saplings were well-fertilized and well–watered, conditions conducive to high rates of photosynthesis, the field site where adults were growing is nutrient poor and subject to drought conditions. In fact, during the year of defoliation, there was a drought during late summer (Song et al. 2013) and gross photosynthesis at the stand level was

greatly reduced (Chapter 3). The reduction in carbon uptake may have impeded the recovery of carbon stores.

A second possibility is that, as adult trees are much larger, refilling stores may take much longer than in small saplings. If carbon store refilling is necessary before normal growth rates can resume, this may explain why defoliation affects growth of larger trees for a longer period of time (Langstrom et al. 1990). Most other studies that observed the replenishment of NSC stores within the same growing season involved saplings or trees less than 2 m tall (Reichenbacker et al. 1996, Palacio et al. 2008, Susiluoto et al. 2010). One study with 36-yr old pines (<5 m tall) did find that needle NSC levels recovered in one growing season (Palacio et al. 2012). But the impact of this defoliation may not have been large as defoliation was not complete, occurred before budbreak, and did not significantly affect growth. In addition, belowground NSC levels were not measured, which can be the organ most affected by defoliation (Li et al. 2002, Landhausser & Leiffers 2012). Another study on 10-yr old *Populus* hybrids also found recovery of NSC levels before the end of the growing season, but not all trees were completely defoliated, and canopy size was restored following refoliation (Kosola et al. 2001).

Less work has been done on the relationship between storage and survival in adult trees, but there is reason to think that a growth-storage tradeoff may occur in adults as well as seedlings. Higher starch levels have been associated with survival in adult trees following defoliation (Webb 1981) and with lower branch mortality following drought (Breda et al. 2006). In addition, several studies have reported that mortality following drought, a soil drainage event, or repeated defoliation was associated with higher growth

rates before the disturbance (McDowell et al. 2010, Levanic et al. 2011, Hultine et al. 2013), suggesting a growth-survival tradeoff. Though the cause of this pattern could not be determined in any case, a tradeoff between growth and storage could be responsible (Hultine et al. 2012). However, the link between survival and a tradeoff between growth and storage has yet to be demonstrated in mature trees, as does any connection between a growth-storage tradeoff and species' habitat preferences as adults.

*NSC levels as growth limitation indicators*

Using NSC levels and dynamics to determine if growth is carbon limited under different conditions has become a common practice. Low or reduced NSC levels and small or reduced seasonal NSC amplitude have been argued to be most likely the result of an increased imbalance between carbon demand for growth and its supply, indicating that growth is currently sink limited or at least nearly carbon saturated (Korner 2003, Hoch et al. 2003). However, patterns of reduced growth that are not accompanied by low NSC levels may also be consistent with carbon limited growth and carbon stress (Sveinbj□rnsson 2000, McDowell 2011, Sala et al. 2012); we have argued this point and have provided experimental evidence that supports this view.

There are two main but related problems with assuming that NSC levels must decline when growth becomes more carbon limited. The first problem is that to be true, accumulation and remobilization of NSC stores must largely be a passive process and growth must always be a higher priority sink. In Chapter 1, we argued that storage is largely an active process, accumulating before growth is carbon saturated. In Chapter 3, using the variation among trees, we demonstrated a positive relationship between radial growth and NSC increment over the growing season. This relationship is not consistent

with passive NSC accumulation as trees that stored more were not more sink limited. This pattern—and therefore active storage—*is* consistent with the idea that NSC levels indicate the degree of carbon limitation. Lower NSC levels were found in trees that were likely the most carbon limited, just as would be predicted. However, our data are not consistent with the idea that NSC levels increase as the result of an imbalance between the carbon supply and demand for growth. In addition, the demonstration of active NSC accumulation—not the result of sink limited growth—is important because *if* NSC accumulates before growth is carbon saturated, then there is no reason to think that *increases* in NSC levels that accompany growth declines cannot also be active.

The second problem with the way NSC levels are used to indicate carbon or sink limitation is that it neglects the possibility that carbon limitation may occur via changes in allocation priorities, independently of changes in net photosynthesis. In Chapter 1, we argued that under certain conditions, storage should increase in priority. Therefore, greater NSC levels and reduced growth may not reflect sink limited growth but rather an increased need for stored carbon. In support of this in Chapter 2, we showed that NSC levels were not reduced along with growth under enhanced carbon limitation. Finally, in Chapter 4 we demonstrated that increased storage belowground coupled with reduced growth in defoliated trees was not the result of sink limitation, but due to shifts in allocation. Shifts in allocation may therefore lead to patterns of higher NSC levels but lower growth rates that can still increase with increasing carbon availability.

We found substantial variation in growth, NSC levels, and NSC increment among trees in a single site and within a single treatment, and it is likely that this level of variation is not unique. When exploring the causes of growth limitation, using among-

tree variation to determine growth-NSC increment, growth-NSC level or growth-NSC pool size relationships within sites, treatments, or species may be a useful addition to comparing average growth and NSC levels. Certainly, it at least can reveal differences in growth and storage patterns that can be obscured by simply comparing average responses. For example, if we consider two sites, we may see that, on average, growth is reduced but NSC levels are increased in site 2 relative to site 1. As we have argued, lower growth may be the result of sink limitation or it may reflect differences in how carbon is partitioned between growth and storage or between storage requirements. As long as there is variation in carbon uptake among trees, the relationship between growth and storage can help to distinguish between these causes. If, in the treatment with lower average growth, storage is negatively or uncorrelated with growth, then the growth reduction may be due to enhanced sink limitation. However, if within this same treatment NSC increment and growth are positively correlated, then growth is likely carbon limited after all.

*A better understanding of sink limitation is needed*

Some of the disagreement about whether carbon limits growth may arise from what is considered to be a sink limitation. Cold temperatures and water stress may both induce sink limitations. The underlying limitations to growth in these cases seem to be imposed directly by the environment. Cold temperatures are believed to inhibit tree growth beyond the treeline by directly reducing the rate of biosynthesis including cell division (Korner 1999). Water stress may directly inhibit growth by reducing cell turgor pressure, restricting cell wall expansion (Hsiao 1973). Under these circumstances, plant growth would not be expected to increase unless these sink-limiting conditions improved.

Alternatively, sink limitations may not be directly imposed by environmental conditions but may arise from developmental constraints (Millard et al. 2001, Korner 2003, Palacio et al. 2008). However, it is not always clear what should qualify as a sink-limiting, developmental constraint. Recent work with Arabidopsis, demonstrates that growth rates may largely be controlled by the plant itself (Smith and Stitt 2007). In response to certain cues, plants can up- or down-regulate the expression and levels of growth promoters and negative regulators. For instance, under salt stress, growth is reduced below what is physically possible and can be enhanced by reduced DELLA protein expression, independent of the alleviation of environmental conditions (Achard et al. 2006). Some researchers might say that growth limited in this way should be considered a sink limitation.

However, many regulatory or developmental constraints on growth could be carbon limitations, at least by the definition that we have supplied (Chapter 1). A good example is the timing of budset which terminates branch growth. If the timing of budset is permanently fixed, this may cause a sink limitation to growth, at least to branch elongation. However, if the timing of budset is flexible and can be altered by carbon availability, then the onset of budset may simply reflect how carbon limited growth is. Trees that are more carbon limited may set buds earlier as perhaps it will take a greater proportion of the growing season to accumulate sufficient NSC to survive the winter and reflush in the spring (for deciduous species). In general, if a developmental or regulatory constraint is dependent on carbon availability, it should not be considered a sink limitation. We therefore need a better understanding of which processes that determine growth rates and timing are sensitive to carbon supply.

*Survival and carbon limitation*

Growth is not the only potentially carbon limited process which can affect carbon sequestration and climate change. Tree survival may also be carbon limited under many circumstances, although mortality mechanisms remain poorly understood (McDowell and Sevanto 2010, Sala et al. 2010). Large-scale forest die-off may becoming more frequent (Breshears et al. 2005, van Mantgem et al. 2009), and if tree mortality continues to increase, it could cause a global reduction in the amount of carbon sequestered in forests (Coomes et al. 2012, Anderegg et al. 2013). However, if the chance of mortality is related to a tree's carbon status, there may be substantial opportunity for survival to be increased by elevated $CO_2$.

We have argued that increased NSC levels and reduced growth, commonly observed in response to stress, may be adaptive strategies for avoiding carbon starvation. While higher NSC levels are associated with survival under low light and severe defoliation, we do not know about other stressors (treeline conditions or drought). Genetic transformation, which is now possible in some tree species, may be a useful tool for exploring the effects of NSC levels on survival under drought and their relationship with growth (Busov et al.2005). Mutants that display increased and decreased growth rates are known in Arabidopsis (e.g. Achard et al. 2006), and transgenic modification has yielded similar results in *Populus* (Busov et al. 2006). While these transgenic trees show marked changes in growth, the effect of these transgenes on storage has not been investigated. In addition, these transgenic trees may be used to explore whether drought causes a physical sink limitation or whether greater growth is actually possible, and if it is, whether this leads to a reduction in NSC levels.

*Conclusion*

Our work suggests that disturbances such as defoliation can cause substantial changes in allocation between growth and storage, and potentially reproduction. These allocation shifts result in enhanced carbon limitation to growth, demonstrating that carbon limitation is a more complex process than often assumed. Carbon limitation may occur independently of reduced carbon uptake, and this fact suggests that tree growth may be carbon limited more often than recently argued. Future investigations of growth limitation under various stressors and in different species or populations should take advantage of the variation among trees and compare the relationship between growth and NSC increment or levels, which can be much more informative than comparing averages of each. In addition, research efforts should address the function of increased storage allocation under stressors such as drought and whether higher NSC levels can increase survival under these conditions.

# Appendix

## Chapter 2

We wanted to determine if defoliated saplings allocated more carbon to storage than growth between harvest dates. Saplings could end up with greater starch level

increments and reduced total biomass in October even if relative allocation between growth and starch ($Conc_{incr}$) was the same. First, this could occur because fully defoliated trees started out with much lower starch levels. The difference in average starch levels between the July and October would have changed more for the fully defoliated trees since the difference between the two starch levels being averaged ($Conc_{incr}$ and $Conc_{july}$) was much larger. Second, because defoliated saplings were smaller in July and October, a difference in starch level increment not due to differential relative starch allocation ($Conc_{incr}$) could occur if the defoliated saplings had grown less in total between July and October, but more relative to final biomass. In this case, the October starch level would be weighted more by the new biomass increment (of higher starch concentration, $Conc_{incr}$, than the July starch biomass).

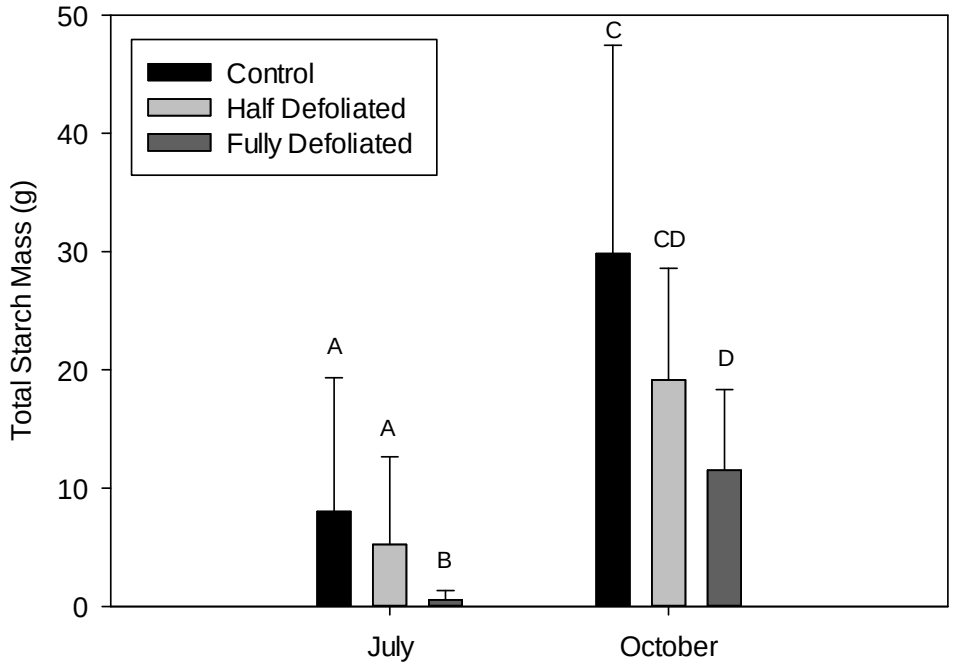



**Figure A2.1: Adjusted averages for total starch mass of woody tissue**. Values are the adjusted averages from the ANCOVA (Principal Component representing initial size used as covariate). Error bars represent 95% confidence intervals. Different letters represent Tukey's HSD between treatments. The starch biomass increment was calculated for each treatment as the difference between these July and October starch biomass adjusted averages.

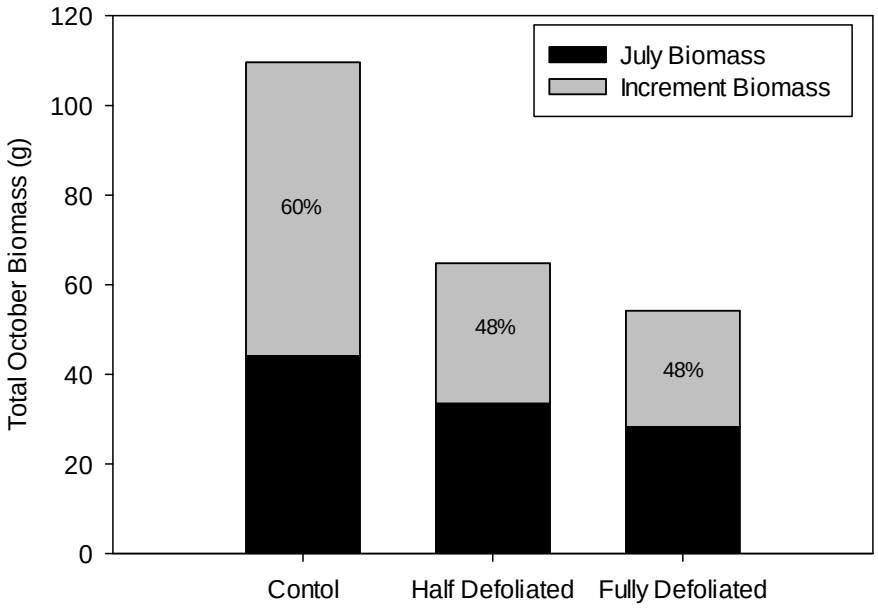



**Figure A2.2**: **Proportions of October woody biomass (*biomass*$_{oct}$) contributed by initial biomass in July (*biomass*$_{july}$) or increment biomass (*biomass*$_{incr}$) which represents new growth.** New biomass accumulated between July and October made up

a greater proportion of October biomass in controls than in defoliated trees (i.e. $biomass_{incr}/biomass_{oct}$ is greater for controls).

## Chapter 3

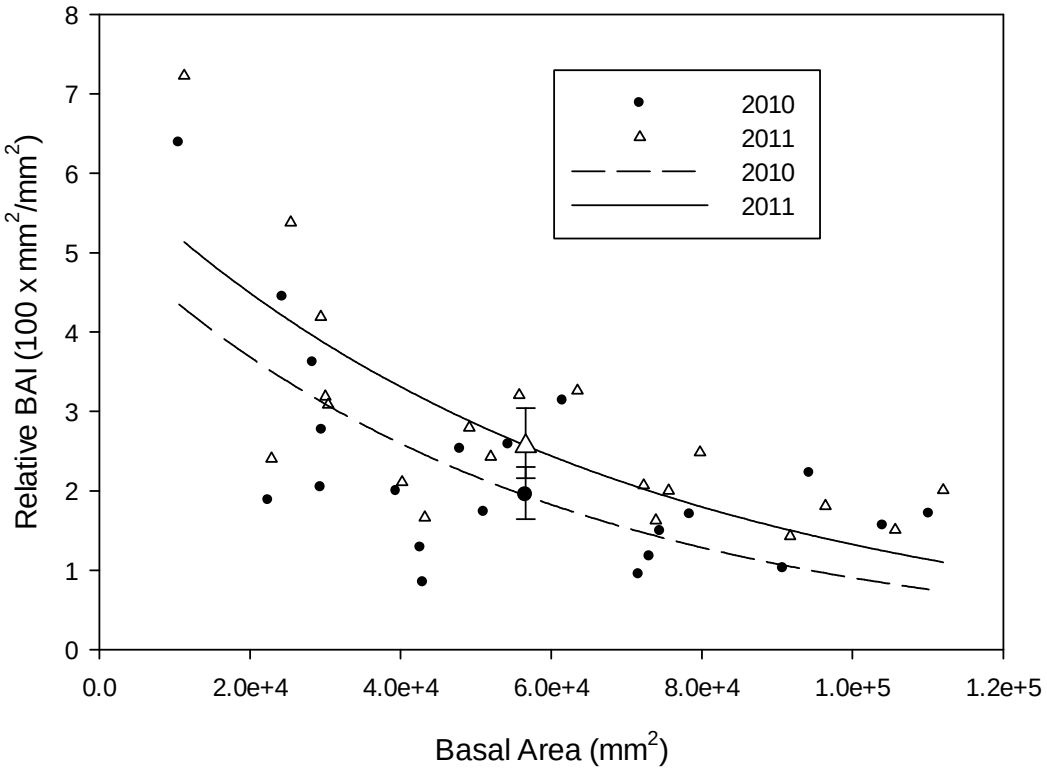



**Figure A3.1**: **Representative relationship between relative basal area increment (rBAI) and initial basal area.** Unadjusted averages are shown with 95% confidence intervals. RBAI, calculated as BAI/ $BA_{initial}$ , was found to be negatively and correlated with $BA_{initial}$. To explore variation in tree growth independent of size, we instead used residual BAI to compare growth among trees. In general, rBAI may not be an appropriate way to control for differences in tree size when the majority of basal area is

not made up of sapwood or living tissue. However, relative BAI may be more informative and appropriate if BAI is divided by initial sapwood area or calculated for trees with little to no heartwood formation.

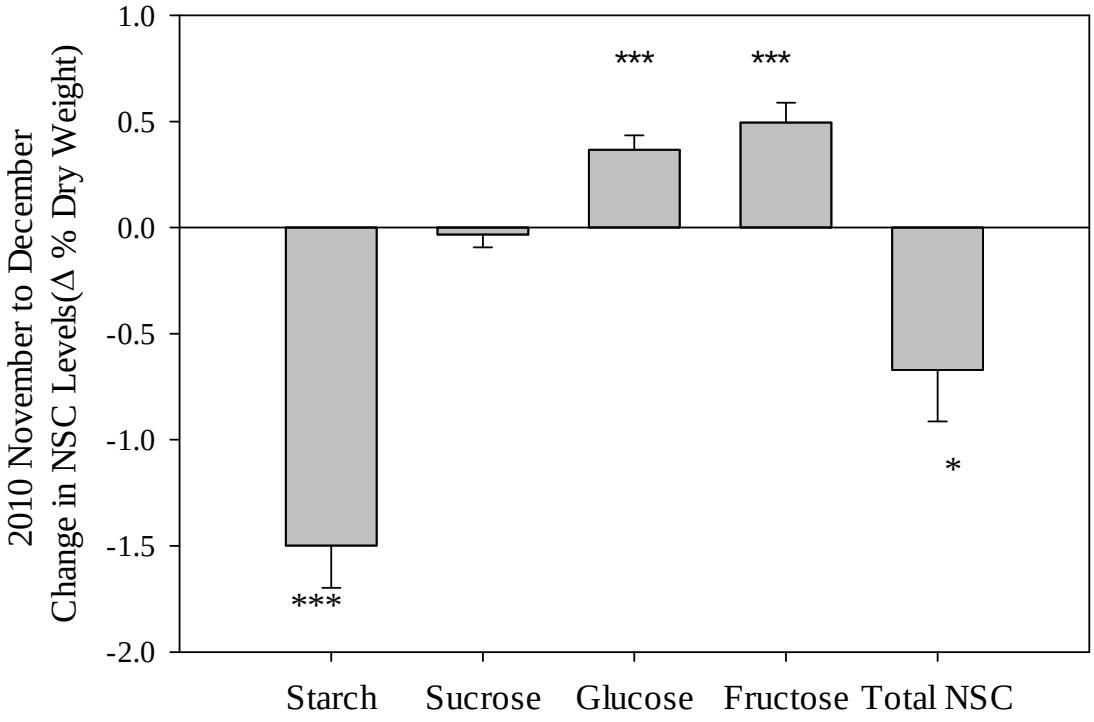



**Figure A3.2**: **Changes in NSC levels from the time of canopy senescence (November) to winter dormancy (December) in 2010**. The slight reduction in total NSC (starch +

soluble sugar) levels was accompanied by a much larger decrease in starch levels and increases in glucose and fructose. Significant changes in NSC levels were determined using paired t-tests with trees that were sampled in both November and December (N=10). Error bars represent standard errors, and significant differences are indicated by asterisks.

## Chapter 4

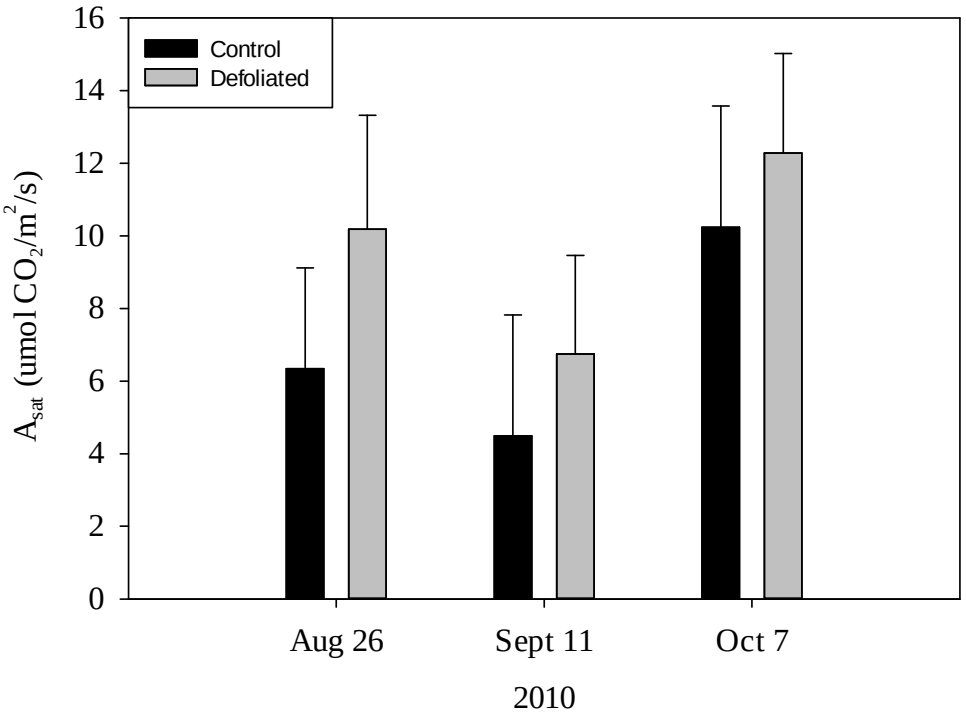



**Figure A4.1**. **Sapling leaf-level photosynthetic rates.** Because leaves from detached branches may behave differently than when intact, we compared our results to measurements on leaves in situ on saplings that we had defoliated in the same site and same year (on July 8, 2010). Measurements were performed on three separate dates, with

September 11 falling near the height of the drought. Defoliated saplings show the same trend as measurements for adults, though again it is not significant for area-based $A_{sat}$ ($F_{1,8.4}$= 0.96, p=0.35).

**Table A4.1. Measurements of 1-yr old branches made in June 2011**

| 2011 | **Control** | **Defoliated** | **ANOVA** | |
|---|---|---|---|---|
| | | | *F* | *p* |
| **# Leaves/stem** | 14.8±2.3 | 10.8±0.7 | 2.79 | 0.13 |
| **Stem diameter (mm)** | *4.6±0.2* | *4.1±0.2* | *3.66* | *0.092* |
| **Stem Length$_{init}$** | 6.9 (4.8-9.7) | 4.7 (3.3-6.7) | 3.06 | 0.12 |
| **Stem Length$_{total}$** | 6.9 (5.0-9.4) | 7.7 (5.6-10.5) | 0.32 | 0.59 |

These measurements correspond to the branches on which acorns were counted. Two length measurements are given: those excluding (init) and including (total) the second flush growth of the defoliated trees that presumably occurred following/concurrent with refoliation in 2010. Averages and standard errors are given for leaves and diameter. Back-transformed averages and 95% CI are given for stem lengths.

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